HIGH-INTENSITY EXERCISE IN HYPOXIA - BENEFICIAL ASPECTS AND POTENTIAL DRAWBACKS

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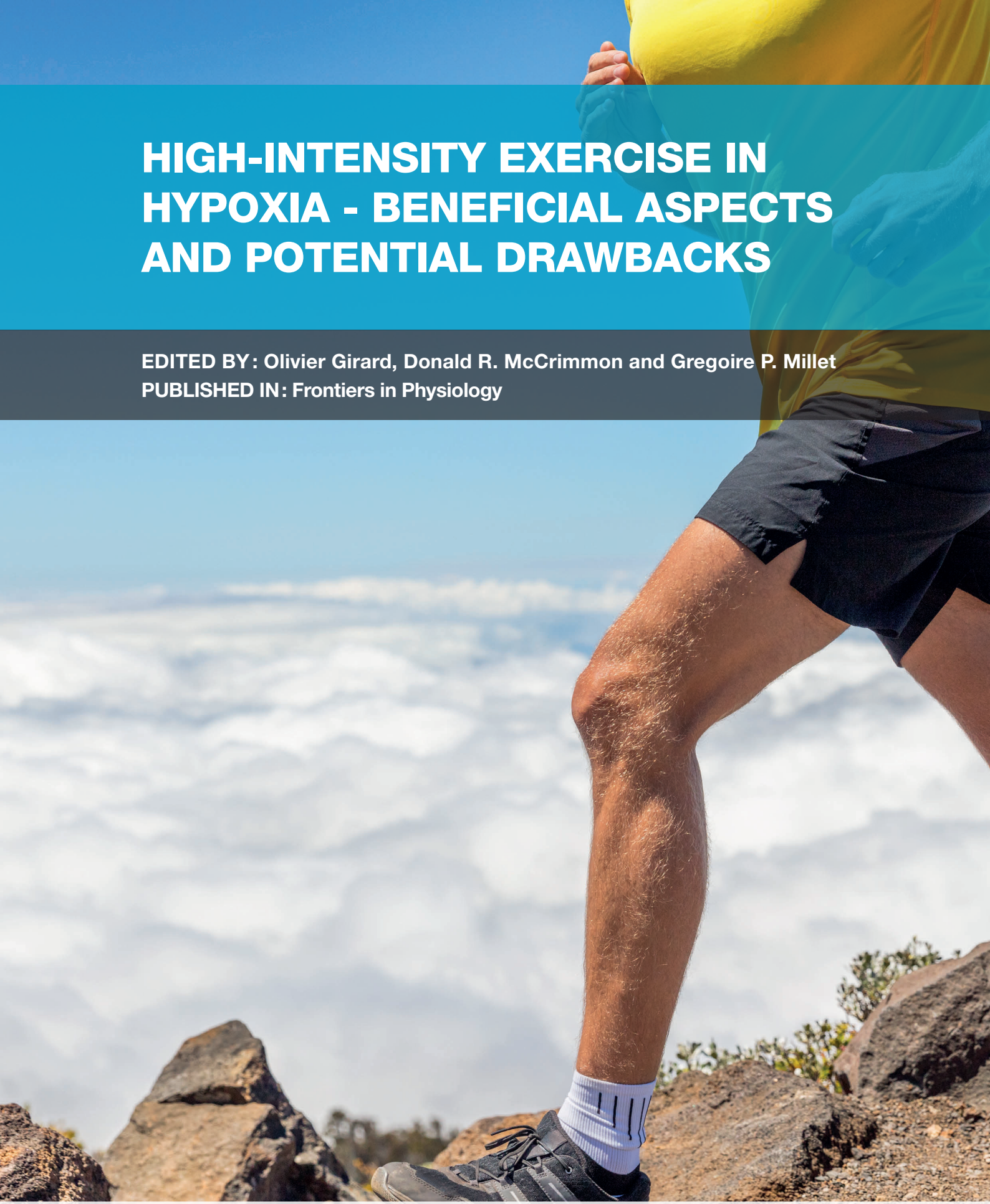

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HIGH-INTENSITY EXERCISE IN HYPOXIA - BENEFICIAL ASPECTS AND POTENTIAL DRAWBACKS

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In the past, 'traditional' moderate-intensity continuous training (60-75% peak heart rate) was the type of physical activity most frequently recommended for both athletes and clinical populations (cf. American College of Sports Medicine guidelines). However, growing evidence indicates that high-intensity interval training (80-100% peak heart rate) could actually be associated with larger cardiorespiratory fitness and metabolic function benefits and, thereby, physical performance gains for athletes. Similarly, recent data in obese and hypertensive individuals indicate that various mechanisms – further improvement in endothelial function, reductions in sympathetic neural activity, or in arterial stiffness – might be involved in the larger cardiovascular protective effects associated with training at high exercise intensities.

Concerning hypoxic training, similar trends have been observed from 'traditional' prolonged altitude sojourns ('Live High Train High' or 'Live High Train Low'), which result in increased hemoglobin mass and blood carrying capacity. Recent innovative 'Live Low Train High' methods ('Resistance Training in Hypoxia' or 'Repeated Sprint Training in Hypoxia') have resulted in peripheral adaptations, such as hypertrophy or delay in muscle fatigue. Other interventions inducing peripheral hypoxia, such as vascular occlusion during endurance/resistance training or remote ischemic preconditioning (i.e. succession of ischemia/reperfusion episodes), have been proposed as methods for improving subsequent exercise performance or altitude tolerance (e.g. reduced severity of acute-mountain sickness symptoms). Postulated mechanisms behind these metabolic, neuro-humoral, hemodynamics, and systemic adaptations include stimulation of nitric oxide synthase, increase in anti-oxidant enzymes, and down-regulation of pro-inflammatory cytokines, although the amount of evidence is not yet significant enough.

Improved O_2 delivery/utilization conferred by hypoxic training interventions might also be effective in preventing and treating cardiovascular diseases, as well as contributing to improve exercise tolerance and health status of patients. For example, in obese subjects, combining exercise with hypoxic exposure enhances the negative energy balance, which further reduces weight and improves cardio-metabolic health. In hypertensive patients, the larger lowering of blood pressure through the endothelial nitric oxide synthase pathway and the associated compensatory vasodilation is taken to reflect the superiority of exercising in hypoxia compared to normoxia. A hypoxic stimulus, in addition to exercise at high vs. moderate intensity, has the potential to further ameliorate various aspects of the vascular function, as observed in healthy populations. This may have clinical implications for the reduction of cardiovascular risks. Key open questions are therefore of interest for patients suffering from chronic vascular or cellular hypoxia (e.g. work-rest or ischemia/reperfusion intermittent pattern; exercise intensity; hypoxic severity and exposure duration; type of hypoxia (normobaric vs. hypobaric); health risks; magnitude and maintenance of the benefits).

Outside any potential beneficial effects of exercising in O_2-deprived environments, there may also be long-term adverse consequences of chronic intermittent severe hypoxia. Sleep apnea syndrome, for instance, leads to oxidative stress and the production of reactive oxygen species, and ultimately systemic inflammation. Postulated pathophysiological changes associated with intermittent hypoxic exposure include alteration in baroreflex activity, increase in pulmonary arterial pressure and hematocrit, changes in heart structure and function, and an alteration in endothelial-dependent vasodilation in cerebral and muscular arteries. There is a need to explore the combination of exercising in hypoxia and association of hypertension, developmental defects, neuro-pathological and neuro-cognitive deficits, enhanced susceptibility to oxidative injury, and possibly increased myocardial and cerebral infarction in individuals sensitive to hypoxic stress.

The aim of this Research Topic is to shed more light on the transcriptional, vascular, hemodynamics, neuro-humoral, and systemic consequences of training at high intensities under various hypoxic conditions.

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EDITORIAL
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Editorial: High-Intensity Exercise in Hypoxia: Beneficial Aspects and Potential Drawbacks

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Keywords: altitude training, deoxygenation, repeated sprint training in hypoxia, resistance training in hypoxia, muscle activation, HIF-1α, ischemic preconditioning, hypoxia

Editorial on the Research Topic

High-Intensity Exercise in Hypoxia: Beneficial Aspects and Potential Drawbacks

RECENT DEVELOPMENTS IN HYPOXIC TRAINING

With the recent development of new altitude training methods (Millet et al., 2013; Girard et al., 2017), the question of the specific central and peripheral adaptations to high-intensity exercise in hypoxia is now crucial. This research topic investigated the beneficial aspects and potential drawbacks of these methods and would undoubtedly be of interest for many exercise physiologists. A total of 16 papers have been accepted, arising from 18 different research groups from 12 countries. Four different main areas have been investigated:

1. High-intensity, continuous exercise in hypoxia.
2. Repeated sprint training in hypoxia.
3. Resistance training in hypoxia.
4. Therapeutic use of hypoxia.

HIGH-INTENSITY, CONTINUOUS EXERCISE IN HYPOXIA

Van Thienen et al. investigated the HIF-1 pathway (from vastus lateralis biopsies) in 11 monozygotic twin pairs who performed an experimental trial in both normoxia and hypoxia. They tested the hypothesis that this pathway and its downstream targets in energy metabolism are regulated in a genotype-dependent manner. A key observation was that hypoxic exercise-induced increment of muscle HIF-1a mRNA content was about 10-fold more similar within monozygotic twins than between the twins. Authors concluded that genetic factors play an important role in the muscular responses to acute hypoxic stress at rest and during exercise and that the regulation of HIF-1a stabilization in acute hypoxia is genotype-dependent.

Townsend et al. computed the critical power (CP) and the work above CP (W') in male cyclists performing time trials in normoxia and at five different altitudes from 250 to 4,250 m. They predicted performance during a high-intensity intermittent test performed in normoxia and in normobaric hypoxia (simulated altitude: 2,250 m). They reported a curvilinear decrease in CP with increase in altitude severity as well as a significant decrease in W′ occurring only at 4,250 m. Practically, this study enables the prescription of equivalent relative intensity interval training workouts in hypoxic conditions compared with normoxia.

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Black et al. explored whether there is a difference in the percentage of VO2max achieved (during a 2-min exhaustive run) in normoxia and hypoxia in 14 middle distance runners. Compared to normoxia, VO2max was lower during a ramp test and VO2 kinetics (greater time constant of the primary phase) were slower in hypoxia. Whereas the runners were unable to reach VO2max during the exhaustive constant work-rate run lasting ∼2 min in normoxia, they were able to achieve the reduced VO2max in a hypoxia despite slower VO2 kinetics.

Torres-Peralta et al. investigated the contribution of central and peripheral mechanisms during exercise to exhaustion in normoxia and hypoxia. Following the exercise to exhaustion, legs circulation was occluded during 10 or 60s for impeding recovery and increasing the metaboreflex. The fact that task failure was apparently not due to muscle peripheral fatigue, but instead primarily resulted from reduction in muscle activation, highlights the importance of central mechanisms.

The same research group Torres-Peralta et al. investigated the role played by different levels of inspired pressure in O2 (P_IO_2) on muscle activation during exhaustive exercise. A unique observation was that the increase in P_IO_2 at exhaustion reduced fatigue and allowed exercise continuation. This study therefore indicates that severe hypoxia induces larger central fatigue (decrease in muscle activation).

REPEATED SPRINT TRAINING IN HYPOXIA

Decrease in convective factors, in turn leading to a reduced training intensity (i.e., not sufficiently intense to stress O_2 delivery and maximized adaptations), is an inherent characteristic of interval-training in hypoxia (IHT) compared with normoxia. To overcome this limitation, the so-called "repeated-sprint training in hypoxia" or RSH has been developed as a new intervention in our laboratory in Lausanne (Faiss et al., 2013a): with exercise intensity being maximal during RSH we have postulated that this would allow a better recruitment of fast-twitch muscle fibers (Faiss et al., 2013a,b). An up-regulation of circulating microRNAs levels was observed only when exercise was performed at high-intensity and high altitude (i.e., and not at lower intensity) and therefore RSH training is based on the repetition of short (<30 s) "all-out" sprints with incomplete recoveries in hypoxia (Vogt et al., 2001; Faiss et al., 2013a). Hence, a lower rate of O_2 delivery to the muscles increases the stress on glycolytic flux, which may stimulate the up-regulation of this energy pathway. Compared with repeated-sprint training in normoxia (RSN), RSH could induce beneficial adaptations at the muscular level, along with improved blood perfusion, which may lead to greater improvements in repeated-sprint ability.

Superior repeated-sprint ability in normoxic conditions has been associated with completion of RSH vs. RSN in cohorts of rugby players (Galvin et al., 2013), well-trained cyclists (Faiss et al., 2013b), cross-country skiers (Faiss et al., 2015), soccer players (Gatterer et al., 2014; Brocherie et al., 2015a), field hockey players (Brocherie et al., 2015b). The effectiveness of RSH was confirmed by a recent meta-analysis based on 9 controlled studies (all published in the past 4 years) showing larger mean performance (Brocherie et al., 2017).

Blood lactate accumulation is higher at a simulated altitude of 4,000 m when compared with more moderate simulated altitudes during repeated treadmill sprints (Goods et al., 2014). In an opinion letter, Scott et al. stated that RSH led to a greater reliance on anaerobic metabolism. This increased metabolic stress is likely to promote peripheral fatigue resistance induced by RSH.

Using near-infrared spectroscopy it was previously reported that prefrontal cortex, but not muscle, oxygenation is impaired when ten, 10-s sprints (with 10 s of rest) are completed at 13 vs. 21% oxygen (Smith and Billaut, 2010). For the first time, Willis et al. compared muscle and cerebral oxygenation trends during a one-off RSH trial performed to exhaustion in normoxia (400 m) and at two different simulated altitudes (2,000 and 3,800 m). There was a continual decrease in convective factors of oxygen delivery (e.g., decreases in pulse oxygen saturation and peak oxygen uptake) with increased hypoxia severity, which was linked with impairment in performance (number of sprints and total work) across conditions. Cerebral deoxygenation demonstrated greater changes at 3,800 m compared with 400 and 2,000 m, as well As well as larger deoxygenation/reoxygenation levels during sprints/recoveries near exhaustion. These results show that central autoregulation (i.e., increase in cerebral perfusion near exhaustion) occurs in order to continue exercise despite limited peripheral and cerebral oxygen delivery, until a certain point of limited diffusion at which protective mechanisms cause exercise cessation.

Sweeting et al. reported that repeated-sprint and single-sprint efforts are compromised at 3,000 m simulated altitude, possibly due to limited muscle O_2 availability during recovery periods. Whilst repeated-sprint and single-sprint efforts were maintained at 2,000 m, the elevated physiological demands at 3,000 m may have been overwhelming.

Girard et al. investigated the neuromuscular adjustments following repeated treadmill sprints at simulated altitudes of 1,800 and 3,600 m or in normoxia. Post-exercise decrease in voluntary strength of knee extensors was greater at 3,800 m than at 1,800 m and normoxia. However, the exercise-induced alterations in rapid torque development were similar between the three conditions.

De Smet et al. investigated if oral nitrate intake influenced buffering capacity and fiber type distribution (vastus lateralis biopsies) after 5 weeks of sprint interval training in hypoxia or in normoxia. Altogether, sprint interval training in hypoxia did not lead to enhanced aerobic or anaerobic endurance exercise performance but oral nitrate supplementation increased the proportion of type IIa muscle fibers. Richardson et al. reported that the improvement in VO_{2peak} and the inflammatory responses (IL-6 and TNFa) were similar after 2 weeks of repeated sprint performed in normoxia or in hypoxia. However, improvement in anaerobic threshold was observed only after RSH.

Hamlin et al. reported the performance changes after six sessions of RSH vs RSN in 19 well-trained male rugby players. These authors confirmed the effectiveness of RSH since repeated sprint performance was enhanced to a larger extent whereas the aerobic performance did not change.

RESISTANCE TRAINING IN HYPOXIA

There is growing research interest focusing on the so-called "resistance training in hypoxia" (RTH) (Scott et al., 2014). A classical reasoning is that, in an O_2-deprived environment, the low partial pressure of O_2 would increase metabolite (e.g., blood [La] and anabolic hormones [e.g., growth hormone]) accumulation, leading to an accelerated recruitment of higher threshold motor units (Manimmanakorn et al., 2013) and a subsequent higher hypertrophy with eventually greater improvements in muscle strength (Scott et al., 2015).

Here, Inness et al. examined the effects of 20 sessions of heavy resistance training performed either in hypoxia (RTH) or in normoxia. RTH induced a larger enhancement in absolute and relative strength as well as 1RM.

Paradis-Deschênes et al. investigated the effects of ischemic preconditioning on knee extensions in strength-trained male vs. female athletes. Males reported a greater peripheral oxygen extraction and greater strength enhancement than females.

That said, a recent meta-analysis (Ramos-Campo et al., 2017) concluded that RTH did not provide significant benefit for muscle size and strength over resistance training in normoxia. This highlights the need for additional research on this burgeoning area.

THERAPEUTIC USE OF HYPOXIA

Potential benefits of using hypoxia exposure therapeutically have been recently suggested for elderly, obese or hypertensive patients (Millet et al., 2016).

Here, Girard et al. postulated that hypoxic walking would be beneficial in obese patients since it might lead to a decreased walking speed and subsequently a lower biomechanical load.

Along the same lines, Pramsohler et al. assessed the physical effort in geriatric patients (age $>$ 65 years) for the same HR response in normoxia or in hypoxia (simulated altitude: 3,000 m). The main benefits of the hypoxic sessions were a lower stress on the locomotor systems for a similar physiological strain than in normoxia.

CONCLUSION

As confirmed by this research topic, high-intensity exercise in hypoxia is a growing area of interest. Ergogenic effect? Stamped! Adaptive mechanisms? More investigation needed! Therapeutic usefulness? The future!

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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ORIGINAL RESEARCH
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Twin Resemblance in Muscle HIF-1α Responses to Hypoxia and Exercise

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Hypoxia-inducible factor-1 (HIF-1) is a master regulator of myocellular adaptation to exercise and hypoxia. However, the role of genetic factors in regulation of HIF-1 responses to exercise and hypoxia is unknown. We hypothesized that hypoxia at rest and during exercise stimulates the HIF-1 pathway and its downstream targets in energy metabolism regulation in a genotype-dependent manner. Eleven monozygotic twin (MZ) pairs performed an experimental trial in both normoxia and hypoxia (FiO_2 10.7%). Biopsies were taken from m. vastus lateralis before and after a 20-min submaximal cycling bout @∼30% of sea-level VO_2max. Key-markers of the HIF-1 pathway and glycolytic and oxidative metabolism were analyzed using real-time PCR and Western Blot. Hypoxia increased HIF-1α protein expression by ∼120% at rest vs. +150% during exercise ($p < 0.05$). Furthermore, hypoxia but not exercise increased muscle mRNA content of HIF-1α (+50%), PHD2 (+45%), pVHL (+45%; $p < 0.05$), PDK4 (+1200%), as well as PFK-M (+20%) and PPAR-γ1 (+60%; $p < 0.05$). Neither hypoxia nor exercise altered PHD1, LDH-A, PDH-A1, COX-4, and CS mRNA expressions. The hypoxic, but not normoxic exercise-induced increment of muscle HIF-1α mRNA content was about 10-fold more similar within MZ twins than between the twins ($p < 0.05$). Furthermore, in resting muscle the hypoxia-induced increments of muscle HIF-1α protein content, and HIF-1α and PDK4 mRNA content were about 3–4-fold more homogeneous within than between the twins pairs ($p < 0.05$). The present observations in monozygotic twins for the first time clearly indicate that the HIF-1α protein as well as mRNA responses to submaximal exercise in acute hypoxia are at least partly regulated by genetic factors.

Keywords: monozygotic twin design, HIF-1, exercise, hypoxia, muscle biopsies

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INTRODUCTION

Whenever the human body is exposed to oxygen deficiency, numerous physiological responses are initiated. Adaptations at both the cardiovascular, respiratory, neurological and skeletal muscle level (Petousi and Robbins, 2014) aim to maintain adequate oxygen uptake and delivery so as to preserve cellular energy homeostasis and tissue integrity. At the level of skeletal muscles, differential mechanisms are involved in the response to either acute or chronic hypoxia. For instance, in acute hypoxic stress fuel selection is shifted from fatty acids to carbohydrates, which increases the ATP yield per molecule of oxygen consumed. Such mechanism not only facilitates energy homeostasis (Hoppeler and Vogt, 2001; Hoppeler et al., 2008; Murray, 2009) but also protects against excessive

mitochondrial production of reactive oxygen species (Zhang et al., 2008; Murray, 2009; Edwards et al., 2010). Furthermore, acute hypoxia also regulates gene expression of several rate-limiting enzymes in the primary energy pathways, i.e., glycolysis, the Krebs cycle, as well as oxidative phosphorylation (Zoll et al., 2006; Murray and Horscroft, 2016). These alterations eventually result in a downregulation of oxidative energy production, vs. upregulation of anaerobic ATP production via glycolysis, aiming to assure adequate rates of sustained ATP-production whenever abundant oxygen supply is lacking, most prominently during exercise (Murray, 2009). The effects of chronic hypoxia on skeletal muscle on the other hand serve to facilitate oxygen diffusion in muscle tissue by stimulation of neovascularization vs. decrease of muscle fiber cross-sectional area, which reduces oxygen diffusion distance (Hoppeler and Vogt, 2001; Deldicque and Francaux, 2013). Furthermore, loss of mitochondrial density and mitochondrial uncoupling decreased ROS production, which might otherwise be exaggerated especially during hypoxic exercise (Murray and Horscroft, 2016).

It is the prevailing opinion that hypoxia-inducible factor 1 (HIF-1) plays a pivotal role in myocellular adaptations to hypoxia (Vogt et al., 2001). HIF-1 is a heterodimeric transcription factor, built of a HIF-1α and a HIF-1β subunit, and serves as an intracellular oxygen-sensor to trigger cellular responses needed to cope with any drop of intramyocellular oxygen tension (Ameln et al., 2005; Mason and Johnson, 2007). In normoxic conditions, in contrast to hypoxia, the HIF-1α subunit is not hydroxylated and is immediately degraded. Conversely, in hypoxia, HIF-1α accumulates in the cytosol and migrates to the nucleus to dimerize with the HIF-1β subunit. The heterodimer so formed can bind to the regulatory domain of different target genes which in turn initiate the concerted cellular response to the hypoxic stress. The importance of HIF-1 in regulation of metabolic genes, including all glycolytic enzymes, *pyruvate dehydrogenase kinase 1* (PDK1) and subunit 4-2 of *cytochrome c oxidase* (COX), but also regulation of angiogenesis is well established (Ameln et al., 2005; Papandreou et al., 2006; Mason and Johnson, 2007; Murray, 2009). Activation of HIF-1 in hypoxic conditions also leads to enhanced mitochondrial autophagy and a decrease in mitochondrial biogenesis and respiration (Zhang et al., 2008). However, data on the inter-individual variability of HIF-1 responses to hypoxia and the role of heritability in HIF-1 regulation in muscle are lacking. Nonetheless, we previously found genetic variants to be important in explaining some specific muscular responses to hypoxia (regulation of maximal oxygen uptake and protein metabolism) (Masschelein et al., 2014, 2015a). Furthermore, epidemiological studies in high-altitude natives to explore the incidence of gene polymorphisms that may be beneficial for survival at high altitude, have provided evidence to indicate that the HIF transcriptional system is associated with some specific loci encoding the erythropoietin and hemoglobin proteins (Simonson et al., 2010; Yi et al., 2010; Petousi and Robbins, 2014). In contrast, the role of genetic factors in modulating the response of HIF-1 in *lowlanders* ascending to altitude is unknown. In fact, qualitative studies on the contribution of genetic factors in exercise performance in hypoxia are scarce (Hennis et al., 2015). To date published literature only supports a potential role of the *angiotensin-converting enzyme insertion* (ACE-I) and *endothelial PAS domain-containing protein 1* (EPAS1) alleles in exercise performance in hypoxia (Montgomery et al., 1998; Masschelein et al., 2015a).

Against this background, the hypothesis driving the current study was that the variability in response of myocellular HIF-1 and its downstream targets implicated in regulation of glycolysis as well as oxidative metabolism, is at least partly explained by genetic factors. To test this hypothesis, we conducted a well-controlled cross-over study (Masschelein et al., 2014, 2015b) in which monozygotic (MZ) twins were exposed to normoxia vs. hypoxia equivalent to ∼5300 m altitude, both at rest and during submaximal exercise. The data presented in this paper for the first time demonstrate that both HIF-1 mRNA and protein expression are upregulated by acute normobaric hypoxia and/or exercise in a genotype-dependent manner.

METHODS

Subjects

The data presented in this paper are original and are part of a larger study in which 13 monozygotic twin brothers ($n = 26$) were enrolled (Masschelein et al., 2014, 2015b). Inclusion criteria on admission were: non-smoking, no history of cardiovascular or respiratory disease, similar physical activity levels within twins, and no residence at altitude >1500 m during 6 months before the study. Mono-zygosity of the twin pairs was confirmed via 8 polymorphic markers (chromosome (chr) 13, GATA30H01 and GATA85D03; chr 18, GATA2E06, GATA64H04, and GATA88A12; and chr 21, GATA163G03, GATA24H09, and GATA71H10). Only 11 of the 13 twin pairs agreed to have muscle biopsies taken during the experiments and were eventually included in the current analyses ($n = 22$; age, 24.4 ± 0.8 years; body weight, 75.9 ± 1.7 kg; VO_2max 55.3 ± 2.1 ml O_2 kg^{-1} min^{-1}). The study protocol was approved by the local ethics committee and was performed in accordance with the Declaration of Helsinki. Subjects gave written consent after being informed in detail of all experimental procedures and passed an a priori medical examination. The twins were instructed to maintain their habitual diet and physical activity levels during the study and to omit exercise for 24 h before each experimental day.

Study Design

Details of the study design have been described previously as this study is part of a greater research project (Masschelein et al., 2014, 2015b). Subjects participated in two experimental days in a normobaric hypoxic facility at 20°C and 50% relative humidity (Sporting Edge, Sherfield on Loddon, UK) with a 2-wk washout period in between. The first experimental trial was in normoxia (NOR; $FIO_2 = 0.209$) and the second one in hypoxia (HYP; $FIO_2 = 0.107$) (see **Figure 1**). The NOR experiment was always done first to exclude possible "memory" effects due to an earlier hypoxic exposure. Breakfast (700 kcal from 84% carbohydrates, 9% fat, and 7% protein) and all later meals and snacks (1880 kcal from 76% carbohydrates, 12% fat,

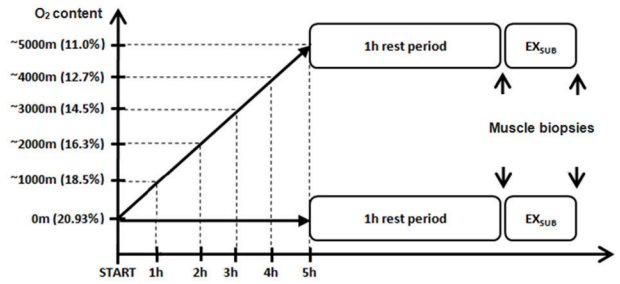

FIGURE 1 | Schematic overview of the experimental study design. Subjects rested for 5 h in either 0.209 FIO_2 (NOR), or while FIO_2 was gradually decreased from 0.209 to 0.107 (HYP). Sixty min after the target FIO_2 was established a needle biopsy sample was taken from the left m. vastus lateralis (pre-ex biopsy, see subsequent figures). Subjects then performed a 20-min submaximal constant-load (1.2 W/kg) exercise (EX_{SUB}) bout on a cycle ergometer after which a second biopsy sample was obtained (post-ex biopsy, see subsequent figures). See Methods for further details.

and 12% protein) and drinks (500 ml of water per 2 h) during both the experimental days were standardized. On each day, twins reported to the 50 m^2 hypoxic facility at 8:00 am and were put in separated compartments within the chamber in order to keep them both ignorant about the experimental events and outcomes occurring in their twin brother. They first rested for 5 h in a comfortable chair in either 0.209 FIO_2 (NOR), or while FIO_2 was gradually decreased from 0.209 to 0.107 (HYP). Thereafter they stayed for an additional 3 h at the target FIO_2 of either 0.209 (NOR) or 0.107 (HYP). Sixty min after the target FIO_2 was established a needle biopsy sample was taken from the left m. vastus lateralis under local anesthesia (1 ml of lidocaine 2 % without adrenaline) through a 5-mm incision in the skin and with the needle tip pointing proximally. Subjects then performed a 20-min submaximal constant-load (1.2 W/kg) exercise (EX) bout on a cycle ergometer (Cyclus II; Avantronic, Leipzig, Germany), corresponding to 50.7 ± 2.3% of VO_2max in normoxia vs. 81.4 ± 3.2% of VO_2max in hypoxia. These exercise modalities (load and duration) were selected after preliminary experiments had shown that 1.2 W/kg for 20 min in similar subjects corresponded to near-maximal exercise tolerance at 0.107 FIO_2. Such workload (∼100 W) corresponds to a normal ascent rate of 300 m per hour in mountaineers (Burtscher, 2004). We also wanted to compare responses to an absolute workload rather than a relative workload. Immediately after exercise (post-ex), another muscle biopsy sample was taken through the same incision as the pre-ex biopsy sample but with the needle pointing distally in the muscle. All muscle samples were quickly frozen in liquid nitrogen and stored at −80°C until biochemical assays were performed.

Western Blot

Details of the immunoblotting procedures were described previously (Deldicque et al., 2010). Briefly, frozen muscle tissue (∼20 mg) was homogenized 3 times for 5 s each with a Polytron mixer (Polytron Technologies, Taoyuan City, Taiwan) in ice-cold buffer [1:10, w/v; 50 mM Tris-HCl, pH 7.0; 270 mM sucrose; 5 mM EGTA; 1 mM EDTA; 1 mM sodium orthovanadate; 50 mM glycerophosphate; 5 mM sodium pyrophosphate; 50 mM sodium fluoride; 1 mM dithiothreitol; 0.1% Triton X-100; and a complete protease inhibitor tablet (Roche Applied Science, Vilvoorde, Belgium)]. Homogenates were then centrifuged at 10,000 g for 10 min at 4°C. The supernatant was collected and immediately stored at −80°C. The protein concentration was measured using a DC protein assay kit (Bio-Rad Laboratories, Nazareth, Belgium). Proteins (30–80 g) were separated by SDS-PAGE (8–12% gels) and transferred to polyvinylidene difluoride membranes. Subsequently, membranes were blocked with 5% nonfat milk for 1 h and then incubated overnight (4°C) with the following antibodies (1:1000): total eukaryotic elongation factor 2 (eEF2) (Cell Signaling, Leiden, The Netherlands) and hypoxia-inducible factor-1α (HIF-1α). Horseradish peroxidase-conjugated anti-rabbit (1:5000) and anti-guinea pig (1:5000) secondary antibodies (Sigma-Aldrich, Bornem, Belgium) were used for chemiluminescent detection of proteins. Membranes were scanned and quantified with GeneSnap and Gene Tools software (Syngene, Cambridge, UK), respectively. The results are presented as the ratio protein of interest/eEF2. All values from the respective condition were reported to the mean value of the first sample (pre-ex) in NOR. One subject was excluded from analysis due to lack of sample.

RNA Extraction and Reverse Transcription

The method used for reverse transcription is described in detail elsewhere (Vincent et al., 2010; Jamart et al., 2011). In brief, total RNA was extracted using TRIzol (Invitrogen, Vilvoorde, Belgium) from 20 to 25 mg of frozen muscle tissue. Total RNA was extracted only in 19 subjects because of the lack of material in 3 individuals. RNA quality and quantity were assessed by spectrophotometry with a NanoDrop (Thermo Scientific, Erembodegem, Belgium). Then 1 g of RNA was reverse-transcribed using a High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Gent, Belgium) according to the manufacturer's instructions.

Real-Time Quantitative PCR Analysis

A SYBR Green-based master mix (Applied Biosystems) was used for real-time PCR analyses using the ABI PRISM 7300 system (Applied Biosystems). Real-time PCR primers were designed for the genes of interest. Thermal cycling conditions consisted of 40 3-step cycles including denaturation for 30 s at 95°C, annealing for 30 s at 58°C, and extension for 30 s at 72°C. All reactions were performed in triplicate. To compensate for variations in input RNA amounts and efficiency of reverse transcription, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and ribosomal protein L4 (RPL4) mRNA were quantified, and results were normalized to these values. These genes were chosen out of 3 normalization genes using the GeNorm applet according to the guidelines and theoretical framework described elsewhere as the Vandesompele-method (Vandesompele et al., 2002). All values from the respective condition were reported to the mean value of the first sample (pre-ex) in NOR.

Statistical Analysis

A 2-way repeated-measures analysis of variance (ANOVA) was used to assess the statistical significance of differences between mean values over time and between conditions (Systat Software, San Jose, CA, USA). When appropriate, a Bonferroni *t*-test was used as a *post hoc* test. In addition, the correlated observations at the level of the twins, together with the individual repeated measures of the oxygen level conditions (NOR vs. HYP) and exercise response (time), were also analyzed using a multilevel mixed-model approach (random effects for time level nested in condition level, nested in twin level; SAS 9.3; SAS Institute, Cary, NC, USA). Because the multilevel mixed-model approach gave the same significant results as the ANOVA, it was chosen to present all data according to the results of the 2-way repeated-measures ANOVA analyses. When the latter analyses revealed a significant difference in protein or mRNA expression, the genetic influence was determined by 2-way repeated-measures ANOVA on one factor, with the twins nested in pairs, and by intra-class correlation coefficients (ICC's; SAS Enterprise Guide 4.3, SAS Institute). F-ratios thus obtained represent the ratio of between-pair over within-pair variability in the induced responses, whereas ICCs provide a quantitative estimate of the similarity within MZ twin pairs and an upper-limit estimate of the genetic component in these responses (genotype × hypoxia and genotype × exercise interaction) (Bouchard et al., 1990). A probability level of $P < 0.05$ was considered statistically significant. All data are presented as means ± standard of the mean (SEM).

RESULTS

Hif-1α Pathway (Table 1 and Figures 2–4)

Compared to NOR, HYP increased HIF-1α protein expression by ∼120% at rest, and by an additional 35% post exercise ($p < 0.05$). Compared to the resting condition in NOR, exercise in HYP raised HIF-1α protein expression about 2.5-fold ($p < 0.05$). HYP, but not exercise, increased HIF-1α mRNA expression by about 30–50% ($p < 0.05$). Irrespective of rest and exercise, HYP stimulated PHD2 as well as pVHL mRNA content by ∼40–50% ($p < 0.05$), whilst PHD1 mRNA content was affected neither by HYP, nor by exercise. Compared to NOR, HYP slightly elevated VEGF-2A mRNA content post ($p < 0.05$) but not pre exercise.

Some of the above average responses were not randomly distributed between subjects. Conversely, some responses were significantly more homogeneous within the twins than between the twins (**Table 1**). Most strikingly, stimulation of HIF-1α mRNA content by the combination of exercise and HYP yielded high similarity between twin brothers (see **Figure 4**). The effect of HYP to stimulate the exercise-induced increment in HIF-1α mRNA content was about 10-fold more homogeneous within twin pairs than between twin pairs ($p < 0.05$), with an ICC as high as 0.91 ($p < 0.05$). By analogy, the effect of exercise to stimulate HIF-1α in HYP, showed about 8-fold higher similarity within the twin pairs (ICC: 0.89, $p < 0.05$) than between the twins. Significant twin resemblance was also found for the effect of HYP to raise HIF-1α protein (ICC = 0.72, $p = 0.05$, **Figure 2**) and mRNA (ICC = 0.79, $p < 0.05$, **Figure 2**) content in resting

TABLE 1 | MZ twin resemblance for muscle protein and mRNA responses to hypoxia and/or exercise.

	Effect of hypoxia				Effect of exercise			
	Pre-ex NOR vs. pre-ex HYP		Post-ex NOR vs. post-ex HYP		Pre-ex NOR vs. post-ex NOR		Pre-ex HYP vs. post-ex HYP	
	ICC	*F*	ICC	*F*	ICC	*F*	ICC	*F*
HIF-1α PATHWAY								
HIF-1α (protein)	0.72*	2.53*	0.45	0.82	0.23	0.29	0.58	1.37
HIF-1α (mRNA)	0.79*	3.77*	0.91*	9.80*	0.44	0.78	0.89*	8.29*
PHD1	0.46	0.86	0.51	1.03	0.85*	5.50*	0.51	1.04
PHD2	0.63	1.68	0.58	1.38	0.70	2.30	0.48	0.94
pVHL	0.52	1.07	0.74#	2.84#	0.52	1.10	0.83*	4.81*
VEGF-2a	0.79*	3.82*	0.67	2.04	0.49	0.94	0.76#	3.10#
GLYCOLYTIC METABOLISM								
PFK-M	0.60	1.50	0.56	1.29	0.67	2.04	0.61	1.58
PDH-A1	0.62	1.63	0.49	0.95	0.77#	3.30#	0.62	1.65
PDK4	0.77#	3.43#	0.78*	3.56*	0.68	2.08	0.11	0.13
LDH-A	0.72#	2.62#	0.45	0.82	0.46	0.85	0.84*	5.17*
OXIDATIVE METABOLISM								
CS	0.48	0.93	0.58	1.39	0.69	2.18	0.70	2.29
COX-4	0.44	0.80	0.59	1.45	0.55	1.20	0.59	1.42
PGC-1α	0.33	0.50	0.74#	2.89#	0.75#	2.98#	0.82*	4.46*
PPAR- γ1	0.77#	3.37#	0.42	0.73	0.46	0.85	0.78#	3.65#
TFAM	0.64	1.77	0.27	0.37	0.59	1.42	0.53	1.13

ICC's and corresponding F-ratios (between/within pair's variance; n = 11) represent similarity within twin pairs for the hypoxia response either pre or post exercise (ex), or similarity within twin pairs for the exercise response during either NOR or HYP. Individual twin-pair responses for the underscored results are presented in ***Figure 4****. *$^{*}P < 0.05$; $^{\#}P < 0.10$.*

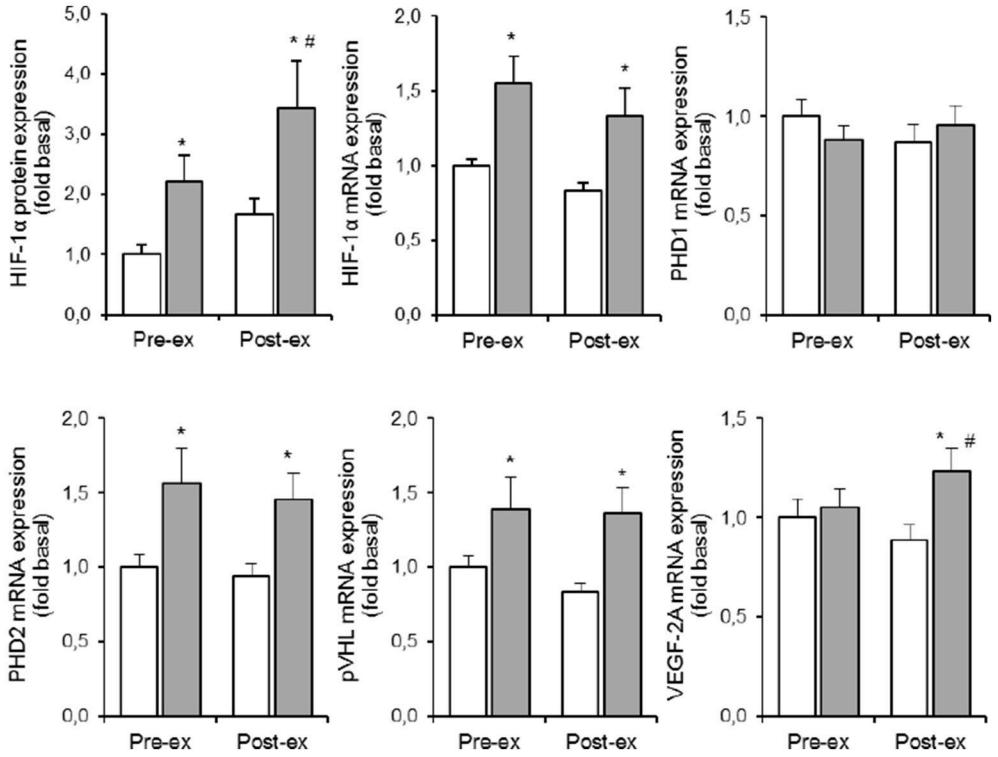

FIGURE 2 | Effect of hypoxia and exercise on the HIF-1α pathway. Data are means ± SEM of 11 twin pairs ($n = 22$) before (pre-ex) and immediately after (post-ex) a 20-min submaximal exercise bout in either normoxia (empty bars) or hypoxia equivalent to ∼5300 m altitude (full bars). HIF-1α, hypoxia-inducible factor 1α; PHD1-2, prolyl hydroxylase domains 1-2; pVHL, Von Lippel-Hindau protein; VEGF-2A, vascular endothelial growth factor-2A. See Methods for further details.*$P < 0.05$ vs. NOR $^{\#}P < 0.05$ vs. pre-ex.

muscle. However, the effect of exercise, alone or in combination with HYP, to stimulate HIF-1α protein content did not yield significant twin resemblance. Furthermore, the exercise-induced increment of pVHL mRNA content in HYP, but not in NOR, was ∼5-fold more similar within twins than between twins (ICC = 0.83, $p < 0.05$).

Glycolytic Metabolism (Table 1 and Figure 5)

Compared to NOR, HYP increased PDK4 mRNA expression ∼12-fold both pre and post exercise ($p < 0.05$; **Figure 3**). In the resting condition in HYP, mRNA levels of PFK-M were ∼20% higher compared to NOR with no additional effect of exercise ($p < 0.05$; **Figure 3**). Neither LDH-A nor PDH-A1 mRNA expression was affected by either exercise or hypoxia (**Figure 5**).

The HYP-induced increased PDK4 gene expression was ∼3.5-fold more similar within twin brothers than between the different twin pairs ($p < 0.05$). Furthermore, exercise in HYP on average did not significantly increase LDH-A mRNA content indeed, yet responses were ∼5-fold more similar within twins than between twins. Overall variability in PFK-M mRNA expression on the other hand, was comparable between subjects with no significant twin resemblance.

Oxidative Metabolism (Table 1 and Figure 6)

HYP increased PPAR-γ1 mRNA expression to the same degree (+60%, $p < 0.05$, **Figure 6**) both at rest and during exercise. Pre exercise PGC-1α mRNA levels were identical in NOR and HYP. However, exercise decreased PGC-1α mRNA expression by ∼15% in NOR ($p < 0.05$), vs. increased PGC-1α mRNA content by ∼15% in HYP ($p < 0.05$, **Figure 4**). TFAM responses to exercise in hypoxia were comparable, which resulted in higher TFAM mRNA content post exercise in HYP compared with NOR ($p < 0.05$, **Figure 4**). Neither exercise nor HYP affected either COX-4 or CS (**Figure 6**) mRNA contents.

The response of PGC-1α mRNA expression to exercise was about 3 to 5-fold more similar within twin pairs than between twin pairs, particularly during exercise in HYP ($p < 0.05$). Conversely, variability of either exercise-induced or hypoxia-induced changes of citrate synthase, COX-4, PPAR-γ, and TFAM mRNA expressions were not significantly different between twins than within twin pairs.

DISCUSSION

In this study we explored the contribution of genetic factors in the myocellular responses of HIF-1α and its downstream targets to acute hypoxia. We exposed monozygotic twin brothers to high altitude (∼5300 m) simulated by means of normobaric

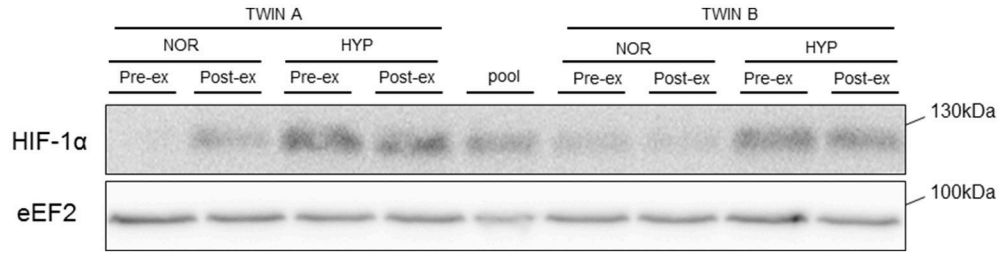

FIGURE 3 | Representative blot of HIF-1α protein. Representative blot of HIF1-α in one monozygotic twin pair before (pre-ex) and immediately after (post-ex) exercise in either normoxia (NOR) or hypoxia (HYP). eEF2 is shown as loading control.

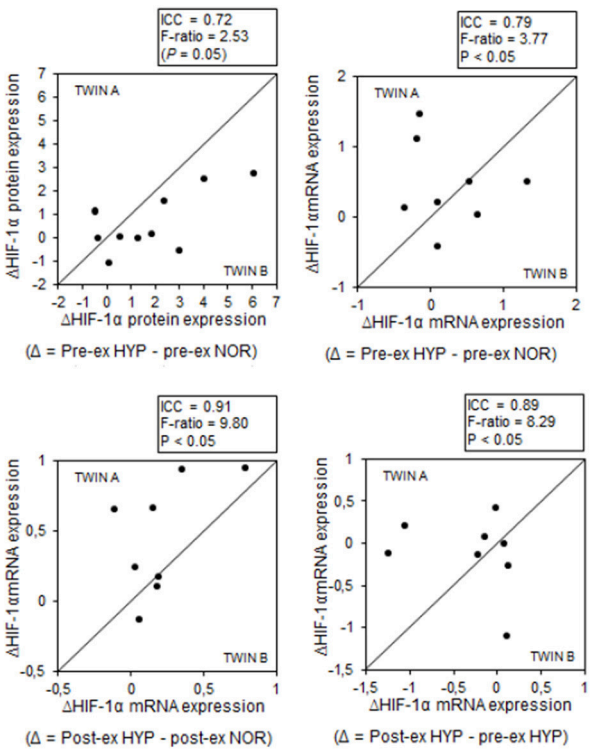

FIGURE 4 | MZ twin resemblance for the effects of hypoxia and exercise on the HIF-1α pathway. Each data point represents the values for one pair of twins (twin A vs. twin B) before (pre-ex) and after (post-ex) a 20-min submaximal exercise bout. Data are hypoxia-induced changes (Δ) and exercise-induced changes for either HIF-1α protein or mRNA expression. Intra-class correlations and corresponding F-ratios (between/within pair's variance) are given in the figure. See Methods for further details.

hypoxia (FIO_2 0.107) and compared the responses with normoxia (FIO_2 20.9%), both at rest and during submaximal exercise. We have previously reported that the current protocol on average decreased VO_2max by ∼40% (range: 25–55%), yet with very high twin resemblance (Masschelein et al., 2015b). Furthermore, we also found hypoxia to modulate protein metabolism at rest and after moderate exercise by increasing markers of protein breakdown, more specifically markers of the autophagy-lysosomal system, including a significant genetic contribution for both hypoxia-induced regulation of REDD1 and microtubule-associated protein 1 light chain 3 (LC3) lipidation (Masschelein et al., 2014). Here we postulated that if heritability is an important determinant of myocellular adaptation to hypoxia, then this must translate into genotype-dependent regulation of HIF-1α during an acute hypoxic challenge. In support of such contention, our current data for the first time clearly demonstrate that acute hypoxia, alone or in conjunction with exercise, substantially increased both muscle HIF-1α mRNA and protein content. Moreover, the HIF-1α increments yielded much higher within-twin than between-twin resemblance, indicating significant genotype impact.

Hypoxia-inducible factor-1α (HIF-1α) plays an important role in the acute responses as well as in the long-term cellular adaptations to oxygen deficiency (Stroka et al., 2001). Upon decreasing intracellular PO_2, HIF-1α translocates to the nucleus to dimerize with the constitutively active HIF-1β. The HIF1 complex so formed eventually acts as a transcription factor for a larger number of genes implicated in regulation of muscle energy metabolism and morphology (Zhang et al., 2008; Luo et al., 2011). In tumor cells, ATP production was impaired and HIF-1α-dependent genes were upregulated whenever intracellular PO_2 dropped below ∼10 mmHg (Richardson et al., 2006; Flueck, 2009). Conversely, muscle cells appear to be more resistant to oxygen deficit because the rate of ATP production is well maintained even at <10 mmHg intracellular PO_2 values inherent to high-intensity muscle contractions (Richardson et al., 1995, 2006; Favier et al., 2015). Conversely, at the very low rates of ATP turnover existing in resting muscle a net arterial PO_2 drop by at least 30 mmHg is needed to significantly decrease intramyocellular PO_2 (Johnson et al., 2005; Richardson et al., 2006). Accordingly, even at an inspired PO_2 as low as ∼75 mmHg corresponding to ∼5500 m altitude, muscle PO_2 is maintained at ∼20–25 mmHg, which is still manifold higher than during maximal exercise in normoxia (<5 mmHg) (Richardson et al., 1995, 2006). Based on the above observations it has been postulated that passive exposure to a low-oxygen environment cannot elicit HIF stabilization (D'Hulst et al., 2013; Favier et al., 2015). Our current observations clearly contradict such conclusion. We did not measure intracellular PO_2, indeed, yet in line with earlier observations (Richardson et al., 2006; Rissanen et al., 2012; D'Hulst et al., 2013) resting arterial oxygen saturation on average decreased from ∼98% in normoxia to

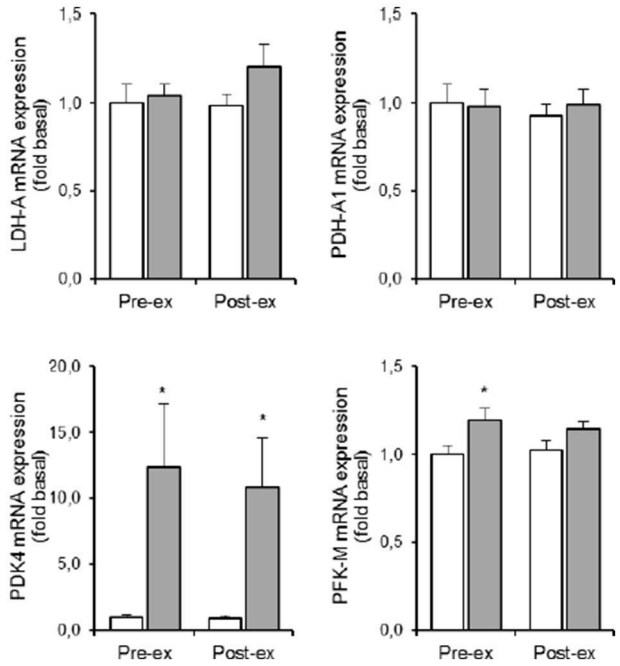

FIGURE 5 | Effect of hypoxia and exercise on glycolytic metabolism. Data are means ± SEM of 11 twin pairs ($n = 22$) before (pre-ex) and immediately after (post-ex) a 20-min submaximal exercise bout in either normoxia (empty bars) or hypoxia equivalent to ∼5300 m altitude (full bars). LDH-A, lactate dehydrogenase-A; PDH-A1, pyruvate dehydrogenase-A1; PDK4, pyruvate dehydrogenase kinase-4; PFK-M, phosphofructokinase muscle-type. See Methods for further details. *$P < 0.05$ vs. NOR.

∼78% in 0.107 FIO_2 (Masschelein et al., 2015b), which should yield intracellular PO_2 values in the range of ∼20–25 mmHg (Richardson et al., 2006). Nonetheless, HIF-1α protein expression on average increased ∼ 2-fold and further increased during exercise (20 min @1.2 W/kg; see **Figure 2**). This submaximal exercise alone did not stimulate HIF-1α protein expression. To the best of our knowledge, this is the first experiment documenting HIF-1α stabilization due to ambient hypoxia in healthy humans at rest. It is difficult to explain the discrepancy between our current and previous observations (D'Hulst et al., 2013). However, besides the rate and magnitude of effected intramyocellular PO_2 decrease, also training status (Lindholm et al., 2014) and history of hypoxic exposure conceivably play a role in regulation of HIF-1α stabilization.

We have previously shown by using near-infrared spectroscopy that normobaric hypoxia equivalent to 5300 m decreased muscle tissue oxygenation by no more than ∼8% compared to normoxic conditions (Masschelein et al., 2014; Van Thienen and Hespel, 2016). Addition of a submaximal cycling exercise bout similar to the present protocol, decreased oxygenation status by an additional ∼12 vs. 5% in normoxia (Masschelein et al., 2014; Van Thienen and Hespel, 2016). These data indicate that the effects of hypoxia and exercise on muscular oxygenation status are additive. Still, tissue oxygenation status measured by near-infrared spectroscopy only partially reflects intramyocellular oxygen tension, which is critical to regulation of HIF-1α. It has been shown that stabilization and expression of HIF-1α is tightly regulated by decreases in intramyocellular PO_2 within the physiological range. Acute exercise reduces myocellular PO_2 to 1/40th of that of inhaled air (PIO_2) while intramyocellular PO_2 values at rest remain at ∼1/5th of the PIO_2 (Richardson et al., 1995). In the conditions of the current study, PIO_2 in hypoxia was ∼75 mmHg which conceivably made intramyocellular PO_2 to drop below 2 mmHg, which is ample to elicit HIF-1α stabilization (Richardson et al., 1995, 2006; Fong and Takeda, 2008). Indeed, due to the specific value of the Michaelis constant of ∼240 μM, the hydroxylase activity of PHD2 is dependent and sensitive to oxygen partial pressures in the range of 0.1–30 mmHg typically occurring in muscle cells at rest and during exercise (Richardson et al., 1995). As the inhibition of PHD2, and thus stabilization of HIF-1α is proportional to the degree of intracellular PO_2 drop (Fong and Takeda, 2008), hypoxia and exercise logically act as additive agents to stimulate HIF-1α protein stabilization and expression via inhibition of PHD2 activity. Furthermore, literature data indicate that HIF-1 is not only regulated by decrease in myocellular PO_2 but also through some humoral factors such as α-ketoglutarate. Plasma α-ketoglutarate concentration increases due to elevated Krebs cycle activity during exercise (Leibowitz et al., 2012). Available evidence indicates that α-ketoglutarate can stabilize HIF-1, probably via inhibition of the PHD2 enzyme (Hou et al., 2014). This provides a mechanism by which exercise *per se*, but not hypoxia, can stimulate HIF-1 activity. Thus, exercise in hypoxia will stimulate HIF-1 activity more than exercise or hypoxia alone, due to Ameln et al. (2005) the synergistic effects of exercise and hypoxia in decreasing myocellular PO_2 and thus activation of PHD2 and (Aragonés et al., 2009) the additional effect of exercise-induced α-ketoglutarate over hypoxia-induced HIF-1 stabilization.

The current hypoxic protocol caused a consistent stimulation of HIF-1α, indeed, yet the responses were highly variable between individuals. Individual changes ranged from −100 to +600% for HIF-1α protein content, vs. no change to +150% for mRNA expression. Interestingly, however, the HIF-1α responses exhibited high twin resemblance. F-ratios indicate that inter-pair variability was about 10-fold greater than intra-pair variability (see **Figure 4**), and hypoxia-induced changes of both muscle HIF-1α protein (ICC = 0.72) and mRNA (ICC = 0.91) contents yielded high MZ twin correlations. In addition, ICCs were substantially higher for the hypoxia effects compared to the exercise effects. This clearly indicates genetic modulation of muscular HIF-1α responses during acute hypoxic stress. Further support for such statement comes from the observation that the hypoxia-induced, but not the exercise-induced changes in mRNA expression of VEGF-2α, a primary downstream target of HIF-1α, were highly similar within twin brothers (ICC = 0.76). In addition, the responses of VEGF-2α were ∼3-fold more variable between twin-pairs than within the pairs. In line with published literature data (Ke and Costa, 2006; Fong and Takeda, 2008), we also observed hypoxia-induced stabilization of muscle HIF-1α to be associated with increased mRNA expression of PHD2 and pVHL. However, for these regulators of HIF-1α degradation we

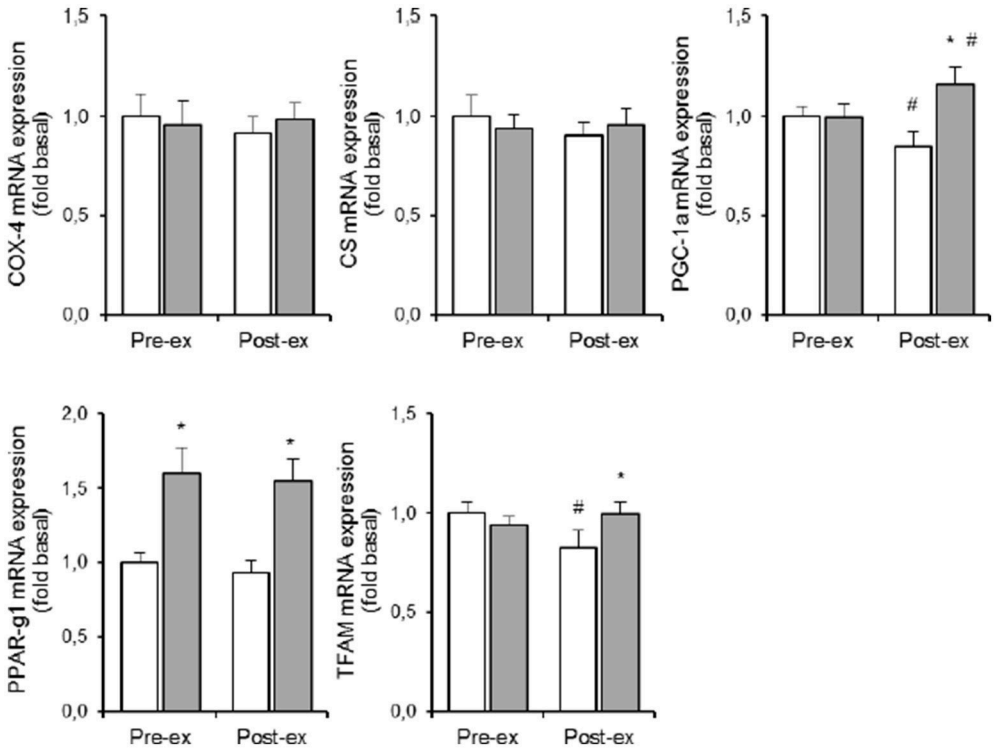

FIGURE 6 | Effect of hypoxia and exercise on oxidative metabolism. Data are means ± SEM of 11 twin pairs ($n = 22$) before (pre-ex) and immediately after (post-ex) a 20-min submaximal exercise bout in either normoxia (empty bars) or hypoxia equivalent to ∼5300 m altitude (full bars). COX-4, cytochrome c oxidase-4; CS, citrate synthase; PGC-1A, peroxisome proliferator-activated receptor gamma, coactivator 1A; PPAR-γ1, peroxisome proliferator-activated receptor gamma-1; TFAM, mitochondrial transcription factor-A. See Methods for further details. *$P < 0.05$ vs. NOR #$P < 0.05$ vs. pre-ex.

could not demonstrate genotype-dependence, which may be at least partly explained by predominant regulation of the HIF-1 pathway at the post-transcriptional level (Aragonés et al., 2009; Formenti et al., 2010). The increase in the mRNA expression of PHD2 and pVHL may seem counter intuitive. However, it has been shown before that acute hypoxia selectively increases expression of both PHD2 mRNA and protein levels in rat glioma cells and in cell cultures (Berra et al., 2003; D'Angelo et al., 2003). This hypoxic upregulation of PHD2 probably acts as a negative feedback loop to immediately stop hypoxic and thus HIF-1 responses in cells once they are re-oxygenated again (Berra et al., 2003; D'Angelo et al., 2003).

Long-term acclimatization to altitude/hypoxia a.o. involves metabolic reprogramming yielding higher glycolytic capacity to compensate for reduced rate of oxidative phosphorylation during episodes of explicit O_2-deficiency, e.g., during contractions (Harris et al., 2002). The pyruvate dehydrogenase (PDH) enzyme-complex channels the output of pyruvate units from glycolysis into either acetyl-CoA formation, or into the lactate dehydrogenase reaction to produce ATP via lactate production. PDK4 inhibits muscle PDH activity by phosphorylation (Harris et al., 2002; Kim et al., 2006; Lee et al., 2012). It has been demonstrated that HIF-1α stimulates PDK4 gene transcription via regulation of the estrogen-related nuclear receptor (Lee et al., 2012). We did not measure ERR activation, yet in keeping with the above pathway of PDK4 activation we found hypoxia-induced HIF-1α stabilization to be associated with a substantial increment of muscle PDK4 gene transcription rate (∼10-fold increased mRNA content; see **Figure 5**). Assuming that repeated hypoxic exposures eventually will upregulate PDK4 enzyme activity, energy provision via anaerobic glycolysis is likely to be facilitated due to inhibition of PDH-A1 activity. Interestingly, the hypoxia-induced change in PDK4 mRNA expression also exhibited high MZ twin resemblance (ICC= 0.77), indicating that PDK4 also is a site of genotype-dependent regulation of acute hypoxic adaptation. Additional support for upregulation of the glycolytic pathway comes from our observation that hypoxia *per se* also slightly elevated muscle PFK mRNA content.

The PGC-1 family of regulated coactivators plays a pivotal role in the control of mitochondrial biogenesis and respiratory function. PGC-1-induced upregulation of mitochondrial mass increases the capacity for oxygen consumption, which in turn leads to drop of intracellular oxygen availability and stabilization of HIF-1 (O'Hagan et al., 2009). However, such chronic adaptive mechanism is irrelevant during acute hypoxia, which in the conditions of the current study did not even elevated muscle PGC-1α mRNA content. However, PGC-1 activity which is largely regulated by posttranslational modifications which in turn tune the activity of a series of downstream targets, including peroxisome proliferator-activated receptor-γ, which

also modulates PDK4 protein expression (Wende et al., 2005). Acute hypoxia here raised PPAR-γ1 mRNA level by ∼60% (**Figure 6**), and again changes were highly similar within the MZ twins (ICC = 0.78, F-ratio = 3.65; $p < 0.05$). This finding adds evidence to indicate that genetic background plays a role in upregulation of anaerobic glycolysis via stimulation of the PPAR-γ ∼PDK4∼PDH pathway in hypoxia.

Because long-term exposure to sever hypoxia decreases mitochondrial volume density in muscle cells (Hoppeler et al., 2003), we also measure some key-enzymes and nuclear transcription factors that are implicated in mitochondrial metabolism and biogenesis. However, 5 h of acute exposure to simulated ∼5300 m altitude changed neither CS nor COX-4 mRNA expression, which is in line with published literature (Edwards et al., 2010; Horscroft and Murray, 2014; Murray and Horscroft, 2016). Also muscle mRNA contents of PGC-1 and TFAM were unchanged, and in addition data variability was small. Hence our current set-up does not allow to evaluate the genetic contribution in hypoxia-induced regulation of oxidative metabolism. Moreover, the timing of the muscle biopsies may have been inadequate to detect elevated mRNA expressions for some of the aforementioned variables.

Because of obvious limitations in finding twin pairs eligible to participate in our invasive study, we chose to recruit only monozygotic twins in order to accumulate a significant number of observations within this specific group. Similar monozygotic twin study designs previously were successful to explore genotype∼environment interactions in phenotype adaptation (Bouchard et al., 1986, 1990). Nonetheless, because we did not include dizygotic twins in the study design, it is not possible to discriminate here between genetic and shared environmental similarity-inducing factors (Bouchard et al., 1990). For MZ twin resemblance results from both true genetic and shared family environmental effects, at least if both members of the pair were raised/lived together. Therefore, twin resemblances reported here probably reflect the upper-limit of heritability. Nonetheless, one should also recognize that data variability inherent to the muscle biopsy procedure *per se* (Van Thienen et al., 2014) as well as rtPCR and Western Blotting assays, confound the true physiological variabilities within and between twins. Therefore, the physiological significance of some upper-limit heritability coefficients may be even higher than could appear from the numerical statistical output. Accordingly, the absence of statistically significant ICC's and/or F-ratio's does not exclude genotype-dependent regulation. For the average effect-size and concurrent data variability for some variables might have been too small, indeed, to allow pertinent correlational analyses to compare within-twin and between-twin variabilities. This study has shown genotype*exercise and genotype*hypoxia interactions to exist for the HIF-1α pathway as well for its downstream transcriptional targets. Future research should aim to identify specific gene variants using genome-wide association studies.

In conclusion, our study provides novel data to prove that genetic factors play an important role in the muscular responses to acute hypoxic stress at rest and during exercise in young healthy individuals. We for the first time clearly show that regulation of HIF-1α stabilization in acute hypoxia is genotype-dependent. This report therefore contributes to a better understanding of the variation in the integrated response to altitude/hypoxia in humans.

AUTHOR CONTRIBUTIONS

RVT and EM: Conception and design of research, prepared figures and manuscript, edited and revised manuscript, approved final version of manuscript. GD: Analyzed results of experiments, edited and revised manuscript, approved final version of manuscript. MT and PH: Conception and design of research, edited and revised manuscript, approved final version of manuscript.

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frontiers in Physiology

ORIGINAL RESEARCH
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Prediction of Critical Power and W′ in Hypoxia: Application to Work-Balance Modelling

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Purpose: Develop a prediction equation for critical power (CP) and work above CP (W′) in hypoxia for use in the work-balance (W'_{BAL}) model.

Methods : Nine trained male cyclists completed cycling time trials (TT; 12, 7, and 3 min) to determine CP and W′ at five altitudes (250, 1,250, 2,250, 3,250, and 4,250 m). Least squares regression was used to predict CP and W′ at altitude. A high-intensity intermittent test (HIIT) was performed at 250 and 2,250 m. Actual and predicted CP and W′ were used to compute W′ during HIIT using differential ($W'_{BALdiff}$) and integral (W'_{BALint}) forms of the W'_{BAL} model.

Results : CP decreased at altitude ($P < 0.001$) as described by 3rd order polynomial function ($R^2 = 0.99$). W′ decreased at 4,250 m only ($P < 0.001$). A double-linear function characterized the effect of altitude on W′ ($R^2 = 0.99$). There was no significant effect of parameter input (actual vs. predicted CP and W′) on modelled W'_{BAL} at 2,250 m ($P = 0.24$). $W'_{BALdiff}$ returned higher values than W'_{BALint} throughout HIIT ($P < 0.001$). During HIIT, $W'_{BALdiff}$ was not different to 0 kJ at completion, at 250 m (0.7 ± 2.0 kJ; $P = 0.33$) and 2,250 m (−1.3 ± 3.5 kJ; $P = 0.30$). However, W'_{BALint} was lower than 0 kJ at 250 m (−0.9 ± 1.3 kJ; $P = 0.058$) and 2,250 m (−2.8 ± 2.8 kJ; $P = 0.02$).

Conclusion: The altitude prediction equations for CP and W′ developed in this study are suitable for use with the W'_{BAL} model in acute hypoxia. This enables the application of W'_{BAL} modelling to training prescription and competition analysis at altitude.

Keywords: high-intensity intermittent exercise, cycling, altitude, hypoxia, fatigue

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INTRODUCTION

The critical power (CP) concept was originally introduced by Monod and Scherrer (1965), and describes the relationship between sustainable power output and duration for severe-intensity exercise. A simple hyperbolic, two parameter model was proposed:

$$W' = t_{lim}/(P - CP) \quad (1)$$

where W′ = total work accumulated above CP until task failure, t_{lim} = duration until task failure, P = power output, and CP = critical power, defined as a rate limited sustainable power output

below which no net expenditure of W′ occurs. Equation (1) can be conceptualized according to a hydraulic model (Margaria, 1976; Morton, 2006), whereby the value W′ progressively depletes during exercise whenever P > CP and reconstitutes when P < CP. Additionally, it can also be seen that if W′ depletes to zero, then t_{lim} also reaches zero and hence task failure is attained. A continuous integral function has been proposed, termed the "work-balance" (W'_{BAL}) model, which mathematically describes the depletion and reconstitution of W′ (Skiba et al., 2012, 2014a, 2015). A differential equation form of the W'_{BAL} has also been proposed (Skiba et al., 2015). As per the abovementioned hydraulic model, the W'_{BAL} models predict that task failure during an intermittent task is attained when W' depletes to 0 kJ. Both versions of the W'_{BAL} model require CP and W′ as input variables, hence accurate estimation of CP and W′ is a prerequisite for computing W'_{BAL}. However, studies have reported that moderate hypoxia decreases CP (Dekerle et al., 2012; Simpson et al., 2015; Shearman et al., 2016) and severe hypoxia decreases both CP and W′ (Valli et al., 2011). Since it is impractical to measure CP and W′ at all possible altitudes, a prediction model which corrects CP and W′ for the effect of hypoxia would permit W'_{BAL} computation at any given altitude.

The mechanistic basis of the two parameter CP model has been extensively studied (Jones et al., 2010). Numerous investigations have demonstrated that CP corresponds to the highest exercise intensity at which pulmonary $\dot{V}O_2$ (Poole et al., 1988; Jones et al., 2008; Vanhatalo et al., 2016), and intramuscular high energy phosphates (Jones et al., 2008; Chidnok et al., 2013), can achieve a steady state. The achievement of steady-state $\dot{V}O_2$ kinetics indicates the net energetic demand of the task can be met by oxidative metabolism (Poole et al., 2016). When the task requirement increases beyond CP the additional energetic demand is supplemented by substrate level phosphorylation (Vanhatalo et al., 2016) which induces non-steady state $\dot{V}O_2$ kinetics, accelerated degradation of high energy phosphates, and accumulation of metabolites involved in peripheral muscle fatigue (Jones et al., 2008; Poole et al., 2016). Since the total amount of work accumulated above CP is terminated at the moment of task failure, the mechanisms leading to task failure itself also determine the value of W′. Recent work examining the etiology of neuromuscular fatigue during high intensity exercise reveals that some combination of both central and peripheral fatigue mechanisms is always present at the moment of task failure, whilst the relative contribution of each depends on the task (Hureau et al., 2016). Observed values for [PCr], [P_i], and [H^+] at task failure have been shown to remain similar despite manipulations in pacing strategy during continuous exercise (Burnley et al., 2010), or recovery duration (Chidnok et al., 2012) and recovery power (Chidnok et al., 2013) during intermittent exercise. Also, following exhaustive high-intensity exercise the magnitude of peripheral fatigue, assessed via twitch interpolation, remains consistent in normoxia and moderate hypoxia (Amann et al., 2006b; Romer et al., 2007). Recently it was demonstrated that changes in peripheral fatigue (assessed via twitch potentiation) was significantly correlated to changes in both [P_i] and [H^+] during exercise when lower limb muscle afferent feedback was impaired using lumbar intrathecal fentanyl (Blain et al., 2016). Collectively, these findings have led to the theory that within a given task, peripheral muscle fatigue may be regulated via group III/IV afferent feedback, which limits central motor drive to the locomotor muscle (Hureau et al., 2016). The existence of such a feedback loop might explain why W′ appears to resemble a fixed capacity within a given task (Broxterman et al., 2015). From a mathematical perspective, a fixed value of W′ allows performance during high-intensity tasks to be predicted using the 2-parameter CP model.

During exercise in hypoxia, convective O_2 transport to the working muscle is reduced (Amann and Calbet, 2008), and multiple studies have reported a significant decrease in CP without a corresponding change in W′ (Dekerle et al., 2012; Simpson et al., 2015; Shearman et al., 2016). If CP is lower in hypoxia, then according to the CP model a given absolute exercise intensity in the severe domain will result in a faster rate of W′ depletion. Previously we reported a large error in modelled W'_{BAL} during intermittent exercise performed in hypoxia when the normoxic CP estimate is used (Shearman et al., 2016). Therefore, CP must either be tested in hypoxia, or estimated from measurements in normoxia. Various studies have examined the effect of increasing altitude on $\dot{V}O_{2max}$ reporting either a linear decrease (Wehrlin and Hallén, 2006; Clark et al., 2007), a curvilinear decrease (Péronnet et al., 1991; Bassett et al., 1999), or a curvilinear interaction between altitude and sea level $\dot{V}O_{2max}$ (MacInnis et al., 2015). To our knowledge, no studies have examined the dose-response effect of increasing altitude on CP and W′. Moreover, a large reduction in W′ was reported at high altitude (5,050 m) (Valli et al., 2011), whereas another study found no change at simulated altitude equivalent to 3,800 m (Simpson et al., 2015). Hence, the approximate threshold altitude where W′ begins to decline remains unclear. The purpose of this study was to examine the dose-response effect of increasing altitude on both CP and W′, and thereafter to develop a prediction equation enabling W'_{BAL} computation in hypoxia. A secondary aim was to compare the integral vs. the differential equation form of the W'_{BAL} model. We hypothesized that CP would decline in a curvilinear fashion commencing from the lowest altitude above sea level (1,250 m) tested, whereas W′ would only begin to decline at altitudes above ≈3,800 m.

Abbreviations: P, Power; W, Watt; CP, Critical power; asymptote of the power–duration relationship; W′, "W-prime" curvature constant of the power–duration relationship; t_{lim}, Time to exhaustion; W'_{BAL}, Work-balance: Amount or balance of W′ remaining; W'_{BALint}, Integral equation form of W'_{BAL} model; $W'_{BALdiff}$, Differential equation form of W'_{BAL} model; τ'_W, Time constant for reconstitution of W′; PCr, Intramuscular phosphocreatine; P_i, Intramuscular inorganic phosphate; H^+, Intramuscular hydrogen ion; ADP_{free}, Intramuscular adenosine di-phosphate free; AMP_{free}, Intramuscular adenosine mono-phosphate free; O_2, Oxygen; $\dot{V}O_2$, Volume of oxygen consumption; HIIT, High-intensity intermittent test; 3TT, 3 min time trial; FiO_2, Fraction of inspired oxygen.

METHODS

Participants

Nine trained male cyclists (mean ± SD; age 34 ± 6 year, 78.1 ± 8.0 kg; $\dot{V}O_{2peak}$ 4.57 ± 0.47 L.min^{-1}) volunteered to participate

in this study, which was approved by the Anti-Doping Lab Qatar Institutional Review Board. All procedures conformed to the standards of the Declaration of Helsinki. Participant inclusion was based on age (18–40 years), training history (2 year minimum cycling training history, 7 $h.wk^{-1}$ minimum average training), and health status (free from injury or illness). All participants were experienced at conducting cycling time trials. Written informed consent was obtained following explanation of the experimental procedures, associated risks, and potential benefits.

Experimental Overview

Participants completed a total of eight testing sessions over a period of 2 months. A minimum of 2, and a maximum of 14 days was specified between any two consecutive lab visits, however, two participants completed one lab visit each outside of this window due to unavoidable personal commitments. The first visit to laboratory involved an $\dot{V}O_{2peak}$ ramp incremental test (30 $W.min^{-1}$) for subject characteristics, followed by a 30 min recovery period and then a 7 min familiarization time trial (TT). Thereafter, on five separate lab visits, participants completed TT's to determine CP and W′ at the following target FiO_2: 0.203, 0.18, 0.159, 0.14, and 0.123, which corresponds to simulated altitudes of 250, 1,250, 2,250, 3,250, and 4,250 m, respectively. The order of condition was counterbalanced according to a latin square design, with participants blinded to the experimental condition. On the remaining two visits, a HIIT at 250 m and 2,250 m was completed. These sessions were not performed after completion of all five TT testing sessions, but rather on the next lab visit immediately following the TT testing at the same altitude. We chose this experimental protocol to minimize the effect of either training or altitude acclimation, on performance during the HIIT. Participants were instructed to avoid strenuous exercise for 24 h prior to each testing session, and to abstain from caffeine and alcohol on the day of testing.

Equipment and Measures

All exercise tests were performed on an electronically braked cycle ergometer (Schoberer Rad Messtechnik, Jülich, Germany) with power was measured at 1 Hz. All simulated altitude conditions were conducted inside a temperature controlled (20°C) altitude chamber (LoxyMed, Berlin, Germany) with stability of target altitude within ±100 m.

Critical Power Testing

The CP test was equivalent to that described and validated by Karsten et al. (2014, 2016). This protocol consists of three TT efforts lasting 12, 7, and 3 min in descending order, interspersed with 30 min of active recovery. We chose to use TTs rather than time to exhaustion (TTe) tests on the grounds that TTs exhibit lower typical error than TTe tests (Paton and Hopkins, 2001) and secondly, recent evidence suggests that CP and W′ parameters estimated from self-paced TTs lead to better prediction of actual TT performance duration then parameters estimated from constant load trials (Black et al., 2015). Participants were blinded to power output, but not duration. Upon completion of the 12 and 7 min TTs, participants exited the altitude chamber within 1-2 min so the first 20 min of the recovery period was always conducted in normoxia. The last 10 min of recovery was conducted inside the chamber at the simulated altitude as specified by the experimental condition.

CP and W′ were initially modelled using three versions of the 2-parameter CP model (1) linear 1/time model, (2) linear work-time model, and (3) nonlinear hyperbolic model (Jones et al., 2008). In each case the standard error of the estimate (SEE) was determined for CP and W′. The lowest SEE for the majority of tests occurred for the linear 1/time model. Therefore, all data analysis used estimates from this model.

High-Intensity Intermittent Test (HIIT)

The HIIT consisted of nine discreet work intervals performed at a target power output predicted to produce task failure during constant load exercise in 5 min according to the 2-parameter CP model:

$$P_5 = (W'/t_{desired}) + CP \quad (2)$$

Where P_5 is power output and $t_{desired}$ is the desired time to task failure (300 s). Interval duration ranged from 40 to 60 s and recovery duration from 30 to 60 s. Immediately following every 3rd work interval a maximal sprint effort (3–5 s) was performed in isokinetic mode at 100 $rev.min^{-1}$. After the 9th work interval, there was a 2.5 min recovery period followed by a self-paced, maximal effort 3 min TT (3TT). Power during all recovery periods was 60 W.

Altitude Prediction and W'_{BAL} Modelling

Mean CP and W′ estimates from each altitude were expressed as a percentage of the values obtained during testing at 250 m. These values were fitted to a 3rd order polynomial using ordinary least squares regression (GraphPad PRISM, USA). Change in W′ with increasing altitude was described using a two-segment linear regression approach since it was expected that no change would occur in W′ until the highest altitude tested (Valli et al., 2011; Dekerle et al., 2012; Simpson et al., 2015; Shearman et al., 2016). Slope one was constrained to 0 ($\%.km^{-1}$) and intercept one was constrained to 100% of baseline level (250 m). Breakpoint, slope and intercept two were left unconstrained. Only measured values for CP and W′ were used to model W'_{BAL} during the intermittent task at 250 m, whereas both the actual measures of CP and W′, and corrected values based on the prediction models, were used to compute W'_{BAL} at 2,250 m.

Modelling of W'_{BAL} during HIIT was conducted using two different equations referred to as the "integral" (W'_{BALint}) model (Skiba et al., 2012) and the "differential" ($W'_{BALdiff}$) model (Skiba et al., 2015). A detailed mathematical derivation from $W'_{BALdiff}$ to W'_{BALint} can be found in the appendix section of Skiba et al. (Skiba et al., 2015). Briefly, the W'_{BALint} model deducts cumulative work expended (or recovered) from the initial W′ to determine W'_{BAL} remaining during an intermittent task. The discharge and reconstitution rate of W'_{BAL} occurs exponentially as shown in Equation (3):

$$W'_{BALint} = W' - \int_0^t W'_{exp} \cdot e^{\frac{-(t-u)}{\tau_{W'}}} \cdot du \quad (3)$$

Where W'_{exp} is the amount of W′ presently expended, and (t-u) is equal to the time in seconds where the athlete is recovering below CP. The time constant for the reconstitution of W′ ($\tau_{W'}$) is a function of the difference between the recovery power and the individual's CP (D_{CP}) according to the following equation (Skiba et al., 2012):

$$\tau_{W'} = 546 \cdot e^{(-0.01 D_{CP})} + 316 \quad (4)$$

The $W'_{BALdiff}$ model treats W′ as a chemical reactant. As per the integral form, W′ reconstitution follows an exponential time course, whilst discharge is strictly linear. However, the time constant is calculated by dividing the starting W′ by D_{CP} rather than fitting data as per equation 4. The discharge of W′ when P>CP using the differential form of the W'_{BAL} is given by:

$$W'_{BALdiff} = W'_0 - \left(W'_0 - W'(u)\right) e^{-\frac{D_{CP}}{W'_0 (t-u)}} \quad (5)$$

Where W'_0 is the initial starting value of W′ prior to a work segment where P>CP, and as above (t-u) is equal to the segment of time where P>CP. Recovery of $W'_{BALdiff}$ occurs during a segment of time when P<CP according to Equation (6):

$$W'_{BALdiff} = W'_0 - W'_{exp}\, e^{-D_{CP} t / W'_0} \quad (6)$$

Where W'_{exp} is the W′ expended during the prior segment in which P>CP. The time course for the entire HIIT is computed by sequentially determining depletion and recovery for each successive segment, where P>CP and P<CP, respectively.

Modelled W'_{BAL} for both the integral and differential equations was computed at 1 Hz throughout the HIIT, but only values at completion of each interval (1 through 9), and the final 3TT, are reported.

Statistical Analysis

Statistical analysis was completed on all data using the Statistical Package for Social Sciences (SPSS) Version 22.0 (SPSS Inc., Champaign, IL). Normality of the data was checked using the Shapiro-Wilk test with ($P < 0.05$) indicating non-normality. Linear mixed modelling was used to examine the fixed effect of altitude on CP and W′, and also to examine fixed effects of model (W'_{BALint} vs. $W'_{BALdiff}$), parameter input (actual vs. altitude corrected CP), altitude (250 vs. 2,250 m), and interval (1 to 9 + 3TT), on modelled W'_{BAL}. Random effects were designated as participant slope and intercept. *Post-hoc* pairwise comparisons were conducted using Sidak's correction and effect sizes were calculated using Hedges' *g*. All pairwise comparisons are reported as mean difference (95% confidence interval: lower, upper; hedges' *g*; *P*-value).

RESULTS

Effect of Altitude on CP and W′

Individual and group mean changes at altitude in CP and W′ are presented in **Figures 1A,B**. At 250 m, mean CP was 269.9 W (95% CI: 250.6, 289.1 W). There was a significant effect of altitude on both CP ($P < 0.001$) and W′ ($P < 0.001$). Compared with 250 m, *post-hoc* comparison showed that CP decreased significantly at 1250 m by 13.0 W (95% CI: 5.6, 20.3; $g = 0.41$; $P < 0.001$), at 2,250 m by 34.9 W (95% CI: 24.8, 44.9; $g = 1.22$; $P < 0.001$), at 3,250 m by 52.3 W (95% CI: 39.9, 64.8; $g = 1.64$; $P < 0.001$), and at 4,250 m by 74.0 W (95% CI: 59.7, 90.1; $g = 2.87$; $P < 0.001$). Mean W′ at 250 m was 17.2 kJ (95% CI: 14.3, 20.1 kJ). Compared with 250 m, no significant differences were found at 1,250 m (−0.5 kJ; 95% CI: −1.6, 2.7; $g = 0.11$; $P = 0.99$), 2,250 m (−0.5 kJ; 95% CI: −2.8, 1.7; $g = 0.12$; $P = 0.99$), or 3,250 m (−1.7 kJ; 95% CI: −4.0, 0.7; $g = 0.39$; $P = 0.3$). At 4,250 m W′ was significantly lower than 250 m (−4.7 kJ; 95% CI: −7.1, −2.3; $g = 1.18$; $P < 0.001$).

Modelling CP and W′ at Altitude

Baseline CP at 250 m was correlated with the magnitude of decline in CP at altitude (expressed as ΔW/km altitude. $r = 0.89$; $P = 0.001$). However, when the decline in CP at altitude was expressed as percent changes, this relationship was not significant ($r = 0.47$; $P = 0.21$). Therefore, to simplify the CP prediction equation, we chose to fit the data as percent changes. Using least squares regression, the decrease in CP with increasing altitude (**Figure 1C**) was best fit to a 3rd order polynomial function ($r^2 = 0.99$) as follows:

$$y = 0.0016x^3 - 0.0157x^2 - 0.027x + 1.0025 \quad (7)$$

Where y = the percent decline in CP from sea level values, and x = altitude in km.

The effect of altitude on W′ was described using a two segment linear model, whereby the intercept and gradient of line one were constrained to 100 and 0%, respectively. The gradient for line 2 was −18.3% (per km) and the breakpoint was 2.76 km. Assuming a mean 95% confidence interval range for W′ measures at all altitudes, this two-segment model predicts W′ measured at sea level, to decline significantly beyond ≈3,500 m (**Figure 1D**).

W'_{BAL} Modelling during Intermittent Task

Figure 2 shows modelled W'_{BAL} during the HIIT for an individual subject. **Table 1** presents group mean data for all W'_{BAL} computations. There was no significant effect of parameter input (actual vs. corrected CP and W′) on modelled W'_{BAL} at 2,250 m ($P = 0.24$). A significant main effect of model (W'_{BALint} vs. $W'_{BALdiff}$) was observed ($P < 0.001$), and also altitude (250 vs. 2,250 m. $P < 0.01$). The altitude by model interaction was significant ($P = 0.02$), where *post-hoc* comparison revealed a significant effect of altitude for the $W'_{BALdiff}$ model only (−0.6 kJ; 95% CI: −0.8, −0.4; $g = 0.86$; $P < 0.001$).

Figure 3 displays computed W'_{BAL} at completion of the 3TT (which concludes the HIIT) for all model variants. $W'_{BALdiff}$ was not different to a value of 0 kJ, which theoretically represents the limit of tolerance during high intensity exercise, at either 250 m (0.7 kJ; 95% CI: −0.9, 2.2 kJ; $g = 0.34$; $P = 0.33$), or 2,250 m for actual model inputs (−1.3 kJ; 95% CI: −3.9, 1.4 kJ; $g = 0.37$; $P = 0.30$), and altitude corrected inputs (−1.1 kJ; 95% CI: −3.6, 1.4 kJ; $g = 0.33$; $P = 0.35$). W'_{BALint} was different to 0 kJ at 2,250 m for both actual (−2.8 kJ; 95% CI: −4.9, −0.7 kJ; $g = 1.03$; $P = 0.02$)

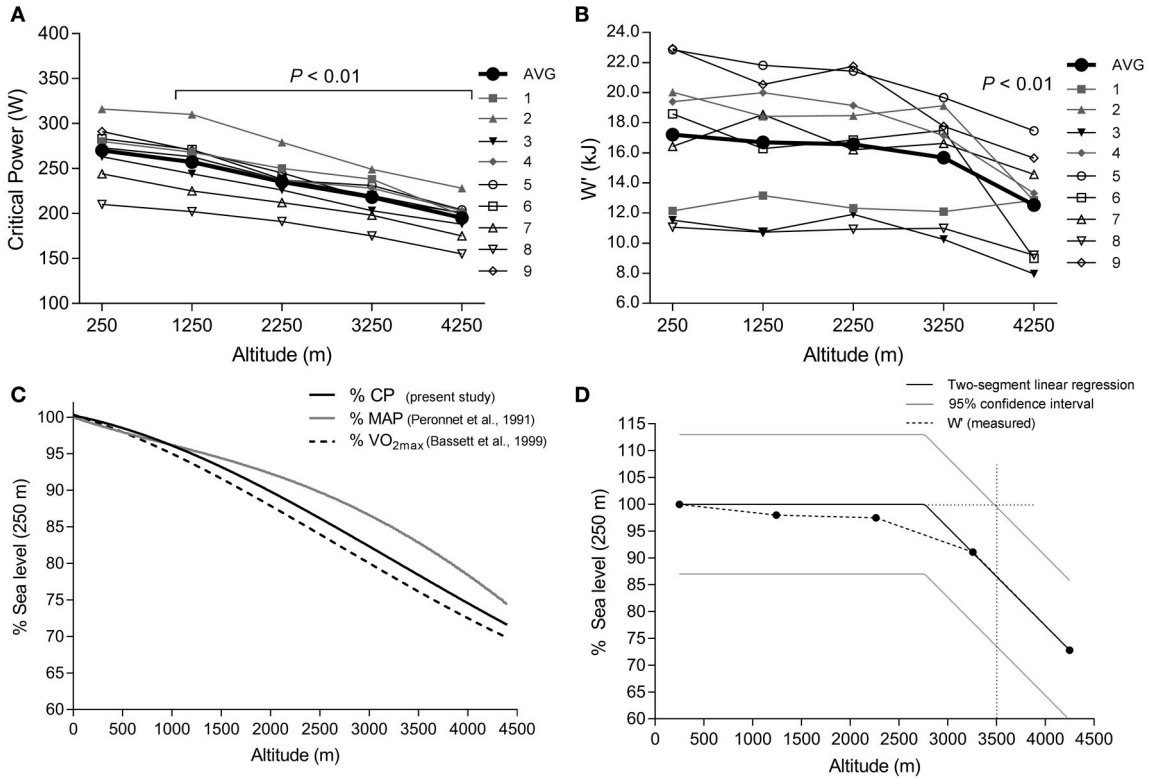

FIGURE 1 | Effect of increasing altitude on group mean and individual subject critical power (A) and W′ **(B)**. Model predicted critical power also showing comparison to maximal aerobic power (MAP) and $\dot{V}O_{2max}$ **(C)**, and W′ **(D)**, expressed as percent of sea level measured values. In **(D)** light gray solid lines represent 95% CI. Intersection of the dotted lines indicates predicted altitude where a statistically significant decline in W′ would occur. $P < 0.05$ indicates significant difference compared to 250 m.

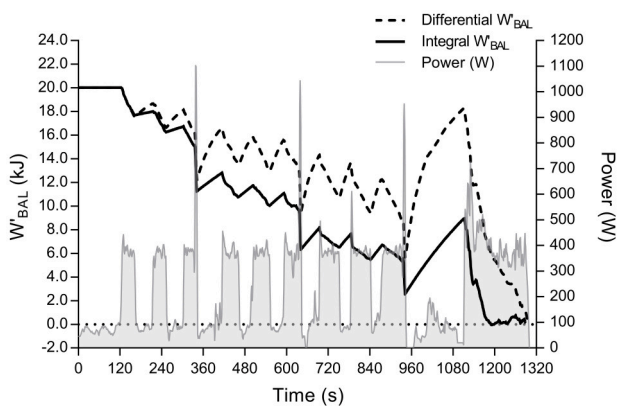

FIGURE 2 | W'_{BAL} responses, showing both integral (solid black line) and differential (dotted line) model computations, during the high intensity intermittent test. Light gray shaded area shows actual power output.

and altitude corrected inputs (−2.6 kJ; 95% CI: −4.5, −0.6 kJ; $g = 1.02$; $P = 0.02$), whilst the difference approached significance at 250 m (−0.9 kJ; 95% CI: −1.9, 0.04 kJ; $g = 0.74$; $P = 0.058$). An example of a field based practical application of the altitude correction to $W'_{BALdiff}$ is shown in **Figure 4**, which was computed from field data during the 2015 Giro d'Italia.

DISCUSSION

Previous studies have quantified the decrease in $\dot{V}O_{2max}$ that occurs at altitude (Bassett et al., 1999; Wehrlin and Hallén, 2006; Clark et al., 2007; MacInnis et al., 2015), however this is the first to examine the effect of altitude on CP and W′. Consistent with data on the dose-response effect of hypoxia on $\dot{V}O_{2max}$ (Bassett et al., 1999; MacInnis et al., 2015), we observed a curvilinear decrease in CP. Secondly, the effect of hypoxia on W′ appears to display threshold type characteristics since there were no significant changes at lower altitudes, whereas a decrease occurred at the highest altitude only (4,250 m). Lastly, we have demonstrated for the first time that a prediction equation can be used in place of actual measurements of CP + W′ at simulated altitude of 2,250 m, to characterize intermittent high-intensity exercise using the W'_{BAL} model.

With increasing altitude, $\dot{V}O_{2max}$ has been shown to decrease linearly (Wehrlin and Hallén, 2006; Clark et al., 2007), or curvilinearly (Bassett et al., 1999; MacInnis et al., 2015). The curvilinear decrease in CP observed in this study (see **Figures 1A,C**) was similar to that for $\dot{V}O_{2max}$ reported by

TABLE 1 | Mean ± SD modelled W'_{BAL} responses at the completion of each interval during the HIIT.

		Intermittent task interval number										
		Initial W′	1	2	3	4	5	6	7	8	9	3TT
250 M												
$W'_{BALdiff}$	Actual	17.2 ± 4.7	15.0 ± 4.1	13.7 ± 3.5	13.4 ± 3.3	11.1 ± 2.7	10.6 ± 2.4	10.8 ± 2.3	8.7 ± 2.1	8.0 ± 1.5	7.4 ± 1.3	0.7 ± 2.0
W'_{BALint}	Actual	17.2 ± 4.7	15.1 ± 4.2	13.5 ± 3.6	12.5 ± 3.3	8.6 ± 2.9**	7.8 ± 2.7**	7.6 ± 2.6**	4.7 ± 2.5**	4.4 ± 2.2**	4.1 ± 2.0**	−0.9 ± 1.3*
2,250 M												
$W'_{BALdiff}$	Actual	16.9 ± 4.0	14.8 ± 3.4	13.6 ± 3.1	13.2 ± 2.9	10.7 ± 2.4	10.2 ± 2.3	10.3 ± 2.2	7.7 ± 2.0†	7.0 ± 1.9†	6.4 ± 1.8†	−1.3 ± 3.5††
	Corrected	16.9 ± 4.0	14.8 ± 3.7	13.6 ± 3.4	13.3 ± 3.2	10.7 ± 2.6	10.3 ± 2.6	10.4 ± 2.6	7.9 ± 2.3	7.2 ± 2.1	6.6 ± 2.0	−1.1 ± 3.3
W'_{BALint}	Actual	16.9 ± 4.0	14.9 ± 3.5	13.4 ± 3.1	12.5 ± 3.0	8.6 ± 2.6**	8.0 ± 2.6**	7.7 ± 2.6**	4.5 ± 2.4**	4.1 ± 2.4**	3.9 ± 2.3**	−2.8 ± 2.8*††
	Corrected	16.9 ± 4.0	14.9 ± 3.5	13.4 ± 3.2	12.6 ± 3.1	8.7 ± 2.8**	8.1 ± 2.9**	7.8 ± 3.0**	4.6 ± 2.8**	4.3 ± 2.7**	4.1 ± 2.7**	−2.6 ± 2.6*

*$P < 0.05$ Integral vs. differential.
**$P < 0.01$ Integral vs. differential.
†$P < 0.05$ 2,250 m vs. 250 m.
††$P < 0.01$ 2,250 m vs. 250 m.

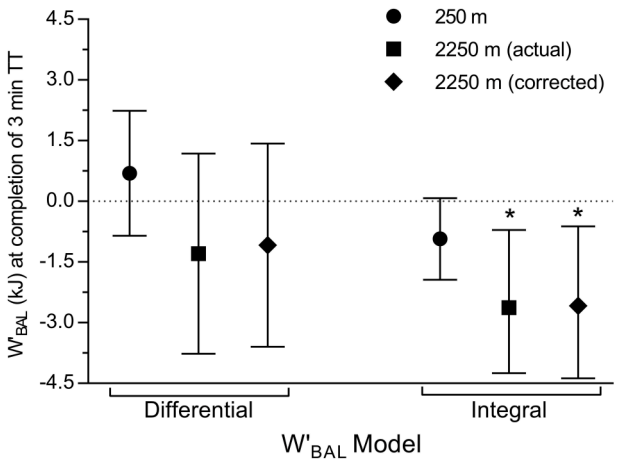

FIGURE 3 | Modelled W'_{BAL} at completion of the 3TT which concludes the HIIT expressed as mean ± 95% CI. Dotted line at $W'_{BAL} = 0$ kJ indicates the theoretical point of exhaustion. *$P < 0.05$ difference compared with 0 kJ.

Bassett et al. (1999). Whilst the effect of hypoxia on the factors that determine $\dot{V}O_{2max}$ are well understood (Wagner, 1996), less is known about the determinants of CP in hypoxia. Traditionally, CP has been considered to reflect a rate limited aerobic energetic supply (Jones et al., 2010). However, it is important to note that $\dot{V}O_2$ at CP is below $\dot{V}O_{2max}$ (Poole et al., 1988; Vanhatalo et al., 2016), and therefore oxidative metabolism at CP cannot be "rate limited." Rather, CP is associated with the highest exercise intensity where a $\dot{V}O_2$ steady state, and muscle "metabolic stability" can be achieved (Poole et al., 1988; Jones et al., 2008; Vanhatalo et al., 2016). Metabolic stability is characterized by minimal disturbance to intramuscular [PCr], [P_i], [H^+], [ADP_{free}], [AMP_{free}] and Gibbs free energy of ATP hydrolysis (Grassi et al., 2011). In hypoxia, a decrease in convective O_2 transport to working muscle occurs (Amann and Calbet, 2008), and the $\dot{V}O_2$ primary component decelerates (Hughson and Kowalchuk, 1995). Since the $\dot{V}O_2$ primary component is considered an "epiphenomenon" of metabolic stability (Grassi et al., 2011), and has been shown to correlate with CP (Murgatroyd et al., 2011), then an O_2 supply limitation on $\dot{V}O_2$ kinetics may impair metabolic stability, and thus explain why CP is reduced in hypoxia (Dekerle et al., 2012; Simpson et al., 2015; Shearman et al., 2016).

In the present study we found no significant differences in W′ at moderate altitudes up to 3,250 m, however a marked reduction (≈27%) occurred at 4,250 m (see **Figure 1B**). These results broadly align with several other investigations examining the effect of differing magnitudes of hypoxia on W′ (Valli et al., 2011; Dekerle et al., 2012; Simpson et al., 2015; Shearman et al., 2016). In recent years improved understanding of the mechanistic basis of W′ has developed. When the exercise intensity increases beyond CP, there is progressive recruitment of type IIx muscle fibers (Copp et al., 2010) and a slowing of $\dot{V}O_2$ uptake kinetics (Brittain et al., 2001). Slower $\dot{V}O_2$ uptake kinetics allows progressive deterioration of muscle metabolic stability to occur which has been demonstrated for both constant load (Jones et al., 2008), and intermittent exercise (Chidnok et al., 2013). The cellular changes associated with failure of metabolic stability are believed to be linked to the emergence of the $\dot{V}O_2$ slow component, and to underlie mechanisms of peripheral muscle fatigue (Grassi et al., 2015). Murgatroyd et al. (Murgatroyd et al., 2011) reported a significant correlation between the $\dot{V}O_2$ slow component magnitude and W′, which suggests the capacity to complete work above CP until the point of exhaustion is ultimately determined by the mechanisms of fatigue contributing to task failure. In moderate hypoxia (FiO_2: ≈0.15), the absolute magnitude of peripheral muscle fatigue, assessed via twitch interpolation, remains similar to normoxia following either constant load work to task failure (Amann et al., 2007), or self-paced TT exercise (Amann et al., 2006a). Additionally, Romer et al. (Romer et al., 2007) found the rate of peripheral fatigue development to increase in hypoxia (FiO_2: ≈0.13) compared with normoxia, but the absolute magnitude remained similar. These findings support the notion that the apparent fixed nature of W′ may be linked to the existence of a peripheral muscle fatigue limit which cannot be surpassed despite

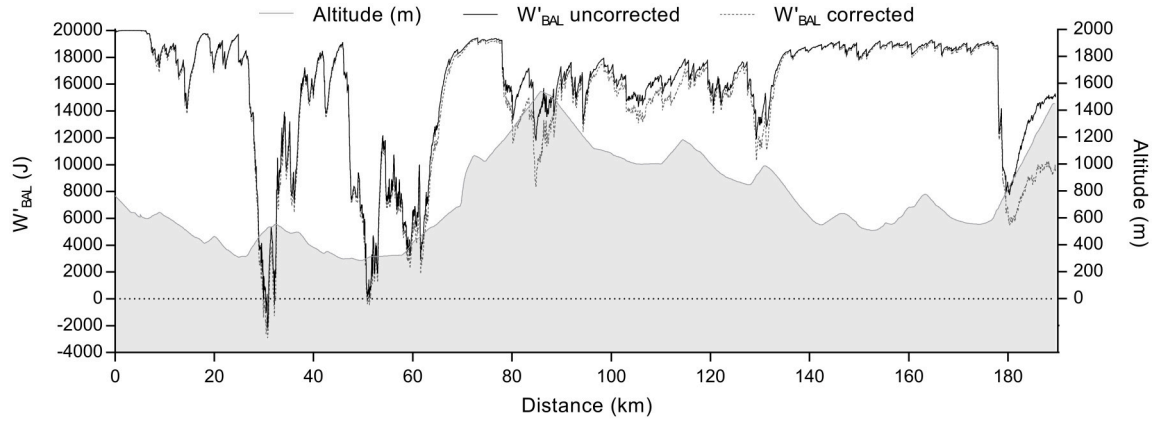

FIGURE 4 | Field data collected during the 2015 Giro d'Italia showing the effect of altitude correction of CP and W′ on modelled $W'_{BALdiff}$ response.

varying experimental conditions, including hypoxia (Amann et al., 2006b; Romer et al., 2007; Poole et al., 2016). However, since hypoxia reduces CP, a given absolute workrate in the severe domain corresponds to a higher intensity *relative* to CP, compared with normoxic conditions. Therefore, according to the CP model, W′ should deplete faster and time to exhaustion would decline. This would be associated with an exacerbated rate of fatigue development in hypoxia, as shown by Romer et al. (Romer et al., 2007). Hence, rather than conceptualizing hypoxia *per se* as the mechanism which exacerbates fatigue, it is the effect of hypoxia on decreasing CP that indirectly leads to a more rapid onset of fatigue, coincident with depletion of W′, at a given absolute workrate.

This study is the first to examine the effect of increasing altitude on W′. Our finding that W′ was markedly reduced only in severe hypoxia (≈27% at 4,250 m) led to the construction of a two-segment linear model (see **Figure 1D**). Using an average confidence interval across all trials, we estimated that a significant decrease in W′ would occur at altitudes beyond ≈3,500 m. Simpson et al. (2015) reported a small decrease in W′ at 3,800 m, but this did not reach statistical significance, whilst Valli et al. (2011) found a large decrease (≈55%) in W′ at 5,050 m. Thus, it appears likely that severe hypoxia reduces W′, yet some uncertainty remains regarding the lowest altitude at which this occurs. Measurement of W′ shows high within-subject variability (Karsten et al., 2014) though, which may confound attempts to accurately determine such a threshold altitude. Valli et al. (2011) suggested the decrease in W′ was consistent with reduced muscle-venous O_2 storage. More recent evidence reveals a decrease in central motor drive in severe hypoxia (≈5,250 m), but no change in moderate hypoxia (≈2,500 m), compared with sea level (Amann et al., 2007). Group III/IV afferent feedback from the locomotor muscles has been suggested to regulate central motor drive (Amann et al., 2006a, 2007), although evidence also suggests that a direct effect of cerebral hypoxia, independent of afferent feedback, may contribute to reduced performance and altered central motor drive in severe hypoxia (Millet et al., 2012). A direct inhibitory effect of cerebral hypoxia on central motor drive might explain the reduction in W′ found in this study and that of Valli et al. (2011), and also the finding that peripheral fatigue is significantly reduced at task failure only in severe hypoxia, but not moderate hypoxia (Amann et al., 2007).

In order to extend the applicability of the constant load CP model to intermittent high-intensity exercise, Skiba et al. (2012) introduced the W'_{BALint} model. This model includes the following assumptions, (1) expenditure of W′ occurs when the power output exceeds CP, (2) reconstitution of the W′ occurs when the power output falls below CP, and (3) the reconstitution of W′ follows a predictable monoexponential time course. The W'_{BALint} model has been validated empirically in normoxia (Skiba et al., 2012, 2014b), whilst a receiver-operator characteristic analysis found subjective rating of exhaustion to occur when the modelled W'_{BAL} fell below 1.5 kJ (Skiba et al., 2014a). Previously, we demonstrated the W'_{BALint} model to be applicable during intermittent high intensity exercise at ≈2,450 m (Shearman et al., 2016). However, this model was only valid when CP and W′ were also measured at the same FiO_2 (Shearman et al., 2016). In the present study, we have shown that a predictable decline in CP occurs with increasing altitude up to 4,250 m (**Figures 1A,C**), and therefore, W'_{BAL} can be calculated in hypoxia using measurements of CP and W′ in normoxia. We found no difference in computed W'_{BALint} or $W'_{BALdiff}$ during intermittent high-intensity cycling at 2,250 m, when either actual measurements of CP and W′ at 2,250 m were used, or predicted values based on measures at 250 m (see **Table 1**).

A modified version of the W'_{BALint} model (Skiba et al., 2012) was recently published (Skiba et al., 2015). This newer model adopted principles of chemical reaction kinetics and takes the form of a differential equation, hence it was referred to as the W'_{BAL} "differential" model. The advantage of a differential equation is that the time constant of W′ recovery does not require prior fitting to empirical data (Skiba et al., 2015), or estimation from Equation 4. The present study though, is the first to directly compare the W'_{BALint} model (Skiba et al., 2012) vs. the $W'_{BALdiff}$ model (Skiba et al., 2015), within a single subject sample (see **Table 1**). We found significantly higher values for

computed $W'_{BALdiff}$ from the fourth interval onwards during the HIIT, at which point W'_{BALint} had declined by ≈50% from initial W′. This difference can largely be explained by the smaller time constant (hence faster recovery kinetics) observed for $W'_{BALdiff}$ vs. the W'_{BALint} model (Skiba et al., 2015). Upon completion of the 3TT at both 250 or 2250 m, $W'_{BALdiff}$ values displayed only small effect sizes and non-significant differences compared with a theoretical criterion of 0 kJ. However, the difference in W'_{BALint} values was moderate at 250 m and large at 2,250 m. This finding contrasts our previous work (Shearman et al., 2016), in which the W'_{BALint} model showed good agreement with a criterion range of $W'_{BAL} = 0 \pm 1.5$ kJ at task failure. In the present study however, we included all-out sprint efforts in addition to self-paced TT exercise during the HIIT. Whilst the $W'_{BALdiff}$ model appeared better suited to the HIIT in this study, the short recovery time constant led to faster W′ reconstitution than reported by Ferguson et al. (2010) for longer recovery durations. Further research is required to understand the limitations of the current mathematical approaches and to develop a more robust model of intermittent exercise.

Since interval training and road cycling competition is highly stochastic in nature, there are limitless permutations of intensity and work to rest ratios. The application of W'_{BAL} approach though, enables analysis of all such permutations within a single unifying mathematical framework. A key justification for the present study was to extend the practical application of W'_{BAL} during dynamic environmental conditions such as a mountain climb in cycling. **Figure 4** presents competition field data from the 2015 Giro d'Italia, during a stage which ascends beyond 1,400 m. The effect of increasing altitude can be seen by comparing the uncorrected CP vs. corrected (for altitude) parameter input into W'_{BAL} model. Interestingly, despite a maximum reported effort on the final hill climb, it appears as though W'_{BAL} is reconstituting since the power is below CP. The failure to deplete W'_{BAL} in this instance likely reflects prolonged accumulation of fatigue mechanisms such as glycogen depletion and/or increasing central fatigue (Thomas et al., 2015), which are not taken into account within the framework of the current models. Glycogen depletion has been shown to decrease the value of W′ (Miura et al., 2000), and fatigue induced inefficiency might decrease CP (Grassi et al., 2015). Accordingly, further research is warranted to develop a robust W'_{BAL} model for a variety of different task requirements. Prolonged endurance exercise would be one such example.

It is well known that endurance exercise performance is reduced upon ascent to altitude (Amann and Calbet, 2008). Secondly, recent progress has been made in the field of mathematical modelling intermittent high-intensity performance, which has practical applications in training load prescription and monitoring (Skiba et al., 2014a, 2015; Shearman et al., 2016). In the present investigation, we report a curvilinear decrease in CP with increasing altitude as well as a significant reduction in W′ occurring only at 4,250 m. The predictable decline in CP, combined with lack of change in W′ up to 3,250 m, enables modelling of W'_{BAL} in hypoxic environments without the requirement for testing at all altitudes. This enables the prescription of equivalent relative intensity interval training workouts in hypoxic conditions compared with normoxia. Whilst we validated use of the altitude correction factor within the W'_{BAL} at 2,250 m, since it is known that W′ contains relatively high typical error (Karsten et al., 2014, 2016), and there may be changes in W′ at higher altitudes, caution is required when interpreting modelled intermittent performance in severe hypoxia above ≈3,500 m.

AUTHOR CONTRIBUTIONS

NT, DN, PS, and JP contributed to experimental concept and design. NT, DN, SR, and JP contributed to data collection and analysis. NT, PS, SR, and JP contributed to manuscript preparation. The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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frontiers in Physiology

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Maximal Oxygen Uptake Is Achieved in Hypoxia but Not Normoxia during an Exhaustive Severe Intensity Run

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Highly aerobically trained individuals are unable to achieve maximal oxygen uptake ($\dot{V}O_{2max}$) during exhaustive running lasting ~2 min, instead $\dot{V}O_2$ plateaus below $\dot{V}O_{2max}$ after ~1 min. Hypoxia offers the opportunity to study the ($\dot{V}O_2$) response to an exhaustive run relative to a hypoxia induced reduction in $\dot{V}O_{2max}$. The aim of this study was to explore whether there is a difference in the percentage of $\dot{V}O_{2max}$ achieved (during a 2 min exhaustive run) in normoxia and hypoxia. Fourteen competitive middle distance runners (normoxic $\dot{V}O_{2max}$ 67.0 ± 5.2 ml.kg^{-1}.min^{-1}) completed exhaustive treadmill ramp tests and constant work rate (CWR) tests in normoxia and hypoxia (F_iO_2 0.13). The $\dot{V}O_2$ data from the CWR tests were modeled using a single exponential function. End exercise normoxic CWR $\dot{V}O_2$ was less than normoxic $\dot{V}O_{2max}$ (86 ± 6% ramp, $P < 0.001$). During the hypoxic CWR test, hypoxic $\dot{V}O_{2max}$ was achieved (102 ± 8% ramp, $P = 0.490$). The phase II time constant was greater in hypoxia (12.7 ± 2.8 s) relative to normoxia (10.4 ± 2.6 s) ($P = 0.029$). The results demonstrate that highly aerobically trained individuals cannot achieve $\dot{V}O_{2max}$ during exhaustive severe intensity treadmill running in normoxia, but can achieve the lower $\dot{V}O_{2max}$ in hypoxia despite a slightly slower $\dot{V}O_2$ response.

Keywords: $\dot{V}O_2$, $\dot{V}O_2$ kinetics, severe intensity, hypoxia, treadmill running

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INTRODUCTION

Middle distance (800–3000 m) running performance is dependent on the speed that an athlete can sustain for the duration of the event. This speed is dependent on the ability of the locomotor muscles to produce power and resist fatigue (di Prampero et al., 1986; Lacour et al., 1990). The relatively high speed sustained throughout middle distance running events results in an energy demand in excess of the maximal aerobic energy yield (~110–120%), as assessed via pulmonary oxygen uptake ($\dot{V}O_2$) and, thus necessitates the integrative contribution from both aerobic and anaerobic pathways (Lacour et al., 1990; Craig and Morgan, 1998; Spencer and Gastin, 2001; Duffield et al., 2005). The 800 m event, for example, requires an ~66 and 34% relative contribution from aerobic and anaerobic metabolism, respectively (Spencer and Gastin, 2001).

The overall energy demand of middle distance running events places these events within the severe, or possibly the extreme intensity domain (Jones and Burnley, 2009). It is assumed that during exercise within the severe or extreme intensity domain, $\dot{V}O_2$ will project exponentially toward the maximal rate of pulmonary oxygen uptake ($\dot{V}O_{2max}$) until $\dot{V}O_{2max}$ is achieved,

or exhaustion occurs (Whipp, 1994; Gaesser and Poole, 1996; Poole and Richardson, 1997; Hill and Ferguson, 1999; Jones and Burnley, 2009). However, research utilizing exhaustive constant work rate (CWR) treadmill running of ∼2 min and highly aerobically trained middle distance runners ($\dot{V}O_{2max} \geq 60$ ml.kg^{-1}.min^{-1}) has found that $\dot{V}O_2$ does not achieve $\dot{V}O_{2max}$ despite sufficient time for the full response to develop (Draper and Wood, 2005a,b; Sandals et al., 2006; James et al., 2007a,b, 2008). Instead, a submaximal steady state $\dot{V}O_2$ is achieved following ∼1 min of exercise with no evidence of a further increase in $\dot{V}O_2$ (Draper and Wood, 2005b).

Previous studies using cross-sectional designs have shown that individuals with a greater $\dot{V}O_{2max}$ achieve a lower percentage of $\dot{V}O_{2max}$ (%$\dot{V}O_{2max}$) during exhaustive CWR treadmill running of ∼2 min (Draper and Wood, 2005a; James et al., 2007a). However, it should be recognized that individuals with a larger $\dot{V}O_{2max}$ typically have faster $\dot{V}O_2$ kinetics (Draper and Wood, 2005b; Kilding et al., 2006; Ingham et al., 2007; Marwood et al., 2010). It is therefore unclear why individuals whom possess a large $\dot{V}O_{2max}$ and faster $\dot{V}O_2$ kinetics achieve a lower %$\dot{V}O_{2max}$ than lesser aerobically trained individuals during exercise of this type.

It is well-known that acute hypoxic exposure results in significant reductions in $\dot{V}O_{2max}$ relative to values obtained in normoxic conditions (Dill et al., 1966; Dill and Adams, 1971; Engelen et al., 1996; Woorons et al., 2005; Calbet et al., 2015), and the decrement in $\dot{V}O_{2max}$ is linearly associated to the fraction of inspired oxygen (FiO_2) (Lawler et al., 1988). Acute hypoxic exposure, therefore, allows the $\dot{V}O_{2max}$ of highly aerobically trained individuals to be artificially and temporarily reduced. Whilst it is recognized that hypoxia may slow $\dot{V}O_2$ kinetics relative to normoxia (Engelen et al., 1996), the magnitude of slowing suggests that $\dot{V}O_2$ kinetics will remain sufficiently fast to permit the manifestation of its full response within <1 min, although evidence from exercise within the severe intensity domain is limited (Heubert et al., 2005). Therefore, hypoxia might provide the opportunity to explore whether highly aerobically trained individuals who are unable to achieve $\dot{V}O_{2max}$ during an exhaustive (∼2 min) CWR treadmill run in normoxia can achieve a hypoxia reduced $\dot{V}O_{2max}$ during a time matched, thus relative intensity matched CWR treadmill run performed in hypoxia.

The purpose of this study, therefore, was to investigate the effect of artificially lowering $\dot{V}O_{2max}$ in trained individuals on their ability to attain $\dot{V}O_{2max}$ during an exhaustive treadmill run. We hypothesized that highly aerobically trained individuals would be unable to attain $\dot{V}O_{2max}$ during a CWR run lasting ∼2 min performed in normoxia, but would be able to achieve a hypoxic reduced $\dot{V}O_{2max}$.

METHODS

Subjects

Thirteen males and one female (mean ± SD: age 21 ± 3 y, height 1.76 ± 0.06 m, mass 66.0 ± 7.0 kg) volunteered for the study. All were trained middle distance runners with an 800 m seasonal best of <130 s. Written and informed consent was obtained prior to data collection. Subjects were instructed to report to all testing sessions in a similar state, following their usual pre-competition routine. The study was approved by the institutional ethics committee.

General Procedures

Subjects completed a laboratory familiarization session which was also used to determine appropriate speeds for the CWR tests. The speeds of the CWR tests were adjusted to ensure exhaustion between 105 and 135 s. All tests were performed in an environmental chamber (Sanyo Gallenkamp, PLC, Loughborough), on the same motorized treadmill (ELG 55, Woodway Gmbh, Weil am Rhein, Germany). Air temperature and humidity were controlled at ∼16°C and ∼40%, respectively. FiO_2 was manipulated to reflect normoxia (FiO_2 0.21) or hypoxia (FiO_2 0.13) by a hypoxic unit (Sporting Edge UK Ltd, Sherfield-on-Lodden).

Following familiarization, subjects visited the laboratory on four occasions to a complete ramp incremental tests and CWR tests, in normoxia and hypoxia. The speed of the treadmill was increased by 0.1 km.h^{-1} every 5 s (1.2 km.h^{-1}.min^{-1}) during the ramp incremental tests, the starting speeds were selected to elicit exhaustion in 8–12 min (Buchfuhrer et al., 1983) in both conditions. The speeds of the CWR tests were based on trial runs completed during the familiarization sessions. If exhaustion was not achieved between 105 and 135 s, the treadmill speed was adjusted and subjects repeated the test on a different day. Trials were randomized to minimize any order effects.

Prior to each CWR run, subjects performed a warm-up on an identical treadmill outside of the environmental chamber. Subjects ran for 5 min at 12 km.h^{-1}, 2 min at 15 km.h^{-1}, and performed 3 × 10 s runs at the speed of the subsequent CWR test interspersed with 30 s of rest. Following the warm-up the subject entered the environmental chamber. Subjects were encouraged to perform light stretching for 2 min. Following the warm-up and stretching routine, subjects straddled the treadmill for 5 min, allowing the belt to move at the required speed for the test. Heart rate (HR) (recorded every 5 s) and breath-by-breath ($\dot{V}O_2$) data were recorded during this period to determine baseline values.

All tests started with the subjects lowering themselves onto the moving treadmill belt. The treadmill was fitted with two handrails, which subjects used to lift themselves onto or clear of the belt. The subject remained in contact with these rails at the start of the test for as long as necessary to reach the required leg speed (typically 2–3 s). The test was stopped when subjects were unable to continue and lifted themselves clear of the treadmill belt.

Data Acquisition

Throughout testing, subjects wore a chest strap and HR was measured using short-range telemetry (810i; Polar Electro Oy, Kempele, Finland), and breathed through a low-dead space (90 ml), low resistance (5.5 cm H_2O at 510 L.min^{-1}) mouthpiece and turbine assembly. Gases were collected continuously from the mouthpiece through a 2 m sampling line (0.5 mm internal diameter) to a quadrupole mass spectrometer (MSX 671: Ferraris

Respiratory Europe Ltd, Hertford, UK) where they were analyzed for O_2, CO_2, Ar and N_2. Expired volumes were determined using a turbine volume transducer (Interface Associates, Alifovieja, US). The mass spectrometer and turbine were calibrated before each test using mixtures of known composition (Linde Gas, London, UK), and a 3 L calibration syringe (Hans Rundolf, KS), respectively. Two identical quadrupole mass spectrometers were used; one was placed outside the environmental chamber to accurately determine the internal environmental conditions, this system was calibrated against outside atmospheric air (20.94% O_2, 0.04% CO_2, 0.93% Argon, and 78.08% N_2) and a normoxic gas bottle (14.99% O_2, 5.01% CO_2, 5.02% Argon, and 74.98% N_2). The second system was placed inside the environmental chamber and was calibrated against the environmental conditions provided by the other mass spectrometer and a gas bottle of known composition; the normoxic gas bottle was used during normoxic testing, and a gas bottle composed of 5% O_2, 5.01% CO_2, 5.02% Argon, and 84.97% N_2 was used in hypoxia. The volume and concentration signals were time aligned, accounting for transit delay in capillary gas and analyser rise time relative to the volume signal. $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$ were calculated for each breath.

Data Analysis

Moving 15 s averages were used to calculate $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}_E$ for every complete 15 s period throughout all tests. $\dot{V}O_{2max}$ was defined as the highest 15 s $\dot{V}O_2$ value attained during the ramp incremental tests, and $\dot{V}O_{2peak}$ was the highest 15 s $\dot{V}O_2$ value achieved during the CWR tests. HR was recorded every 5 s and the highest value achieved during the ramp incremental test was taken as maximum HR (HR_{max}) and the highest value recorded during the CWR exercise was the peak HR (HR_{peak}).

The breath-by-breath $\dot{V}O_2$ data from the CWR tests were initially examined to exclude errant breaths caused by coughing, swallowing, etc., and values lying more than 4 SD from the local mean were removed. Subsequently, the breath-by-breath data were converted to second-by-second data using linear interpolation and time aligned to the start of the test. The first 15 s of data were removed to account for the cardio-dynamic phase (Murias et al., 2011). A single exponential model was used to characterize $\dot{V}O_2$ kinetics as described in the following equation:

$$\dot{V}O_2(t) = \dot{V}O_2 \text{ baseline} + A(1 - (e^{-(t-\delta)/\tau})) \quad (1)$$

where $\dot{V}O_2$ (t) represents the absolute $\dot{V}O_2$ at a given time (t), $\dot{V}O_2$ baseline is the average of the $\dot{V}O_2$ measured over the final 120 s of quiet standing, A is the asymptotic amplitude, τ is the time constant of the exponential response and δ is a delay. No parameters were constrained.

Statistical Analysis

Data were tested for normality (Duffy and Jacobsen, 2001) and was found to be normally distributed. Two-way (test × condition) repeated measures ANOVA was employed to determine the effect of hypoxia on $\dot{V}O_2$, minute ventilation ($\dot{V}_E$), ventilatory equivalents (i.e., $\dot{V}_E/\dot{V}O_2$, $\dot{V}_E/\dot{V}CO_2$) and HR. *Post hoc* t-tests with Bonferroni correction were used to explore the origin of any significant interaction effect. Paired t-tests were used to explore differences in estimates of the modeled $\dot{V}O_2$ data in normoxia and hypoxia. Pearson's Product Moment Correlation was used to investigate the relationship between $\dot{V}O_{2max}$, CWR running speed, and the % $\dot{V}O_{2max}$ achieved during the CWR tests. The relationship between the difference in running speed and the difference in % $\dot{V}O_{2max}$ achieved during the normoxic and hypoxic CWR tests was also investigated. Statistical significance was set at $P < 0.05$. Data are presented as mean ± SD unless otherwise stated.

RESULTS

The $\dot{V}O_{2max}$ measured in the normoxic ramp incremental test was 4.40 ± 0.42 L.min^{-1} (67.0 ± 5.2 ml.kg^{-1}.min^{-1}) and HR_{max} was 185 ± 7 bpm. Hypoxia reduced $\dot{V}O_{2max}$ to 2.97 ± 0.27 L.min^{-1} (45.1 ± 3.0 ml.kg^{-1}.min^{-1}; $P < 0.001$) and HR_{max} to 181 ± 6 bpm; $P < 0.05$).

The average speed utilized for the normoxic CWR trials was 22.0 ± 1.0 km.h^{-1} which resulted in a trial duration of 114 ± 11 s (range: 100 s to 130 s). The speed of the hypoxic CWR trial was performed at a significantly slower speed (20.5 ± 1.0 km.h^{-1}; $P < 0.001$) to ensure a similar duration of trial between conditions. The duration of the hypoxic CWR trial (114 ± 11 s, range: 105 s to 135 s) was not significantly different to the duration of the normoxic CWR trial (114 ± 5 s, range: 105 s to 125 s) ($P > 0.05$). Normoxic $\dot{V}O_{2max}$ was not achieved during the normoxic CWR trial (3.79 ± 0.47 L.min^{-1}; 86 ± 6% $\dot{V}O_{2max}$; $P < 0.05$; **Figure 1**).However, subjects attained hypoxic $\dot{V}O_{2max}$ during the hypoxic CWR trial (3.02 ± 0.30 L.min^{-1}; 102 ± 8%; $P > 0.05$; **Figure 1**). $\dot{V}O_{2max}$ was inversely associated with %$\dot{V}O_{2max}$ achieved during the normoxic ($r = -0.64$, $P < 0.05$) and hypoxic ($r = -0.68$, $P < 0.01$) CWR trials, and when the normoxic and hypoxic trials were combined ($r = -0.85$, $P < 0.001$; **Figure 2**). Condition-specific HR_{max} was attained during normoxic (189 ± 7 bpm) and hypoxic (181 ± 7 bpm) CWR trials ($P > 0.05$). The parameters of the modeled $\dot{V}O_2$ data are presented in **Table 1**. No relationships were observed between speed and % $\dot{V}O_2$ achieved during the CWR trials performed in normoxia ($r = 0.34$, $P > 0.05$), hypoxia ($r = -0.16$, $P > 0.05$), or the difference in speed and $\dot{V}O_2$ between the normoxic and hypoxic CWR trials ($r = -0.05$, $P > 0.05$).

No significant interaction effect was observed for $\dot{V}_E$ ($P > 0.05$) with no significant main effect for condition (normoxia, 137.3 ± 17.5 L.min^{-1}; hypoxia 130 ± 16.2 L.min^{-1}; $P > 0.05$), but a significant main effect for test (ramp, 128.0 ± 16.6 L.min^{-1}; CWR, 139.2 ± 16.0 L.min^{-1}; $P < 0.001$). There was a significant interaction effect for $\dot{V}_E/\dot{V}O_2$ ($P < 0.05$) with significant main effects for condition (normoxia, 35.3 ± 6.7 L.min^{-1}; hypoxia 43.7 ± 4.7 L.min^{-1}; $P < 0.001$) and test (ramp, 128.0 ± 16.6 L.min^{-1}; CWR, 139.2 ± 16.0 L.min^{-1}; $P < 0.001$). There was a significant interaction effect for $\dot{V}_E/\dot{V}CO_2$ ($P < 0.01$) with significant main effects for condition (normoxia, 28.2 ± 3.7 L.min^{-1}; hypoxia 31.6 ± 6.0 L.min^{-1}; $P < 0.05$), but no significant difference for test (ramp, 29.8 ± 5.0 L.min^{-1}; CWR, 26.2 ± 3.4 L.min^{-1}; $P > 0.05$).

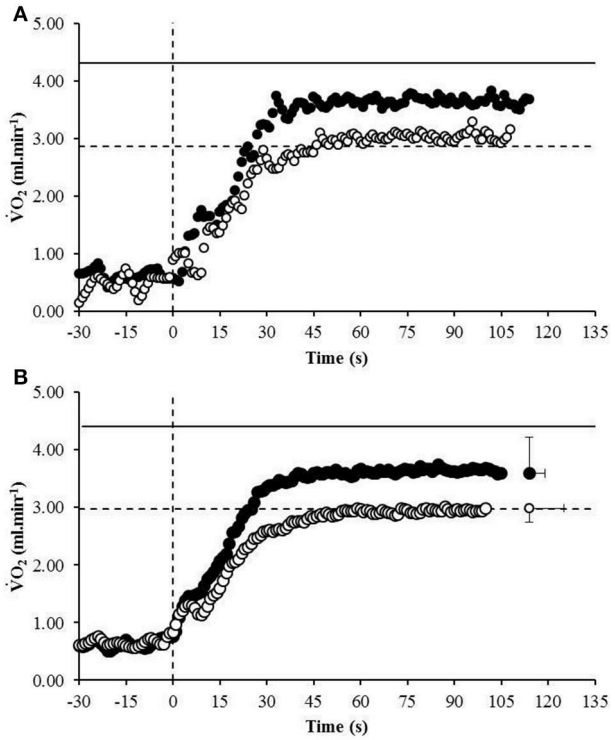

FIGURE 1 | The $\dot{V}O_2$ response of a representative participant (A) and the group mean **(B)** to the normoxic CWR test (black circles) and hypoxic CWR test (white circles). The $\dot{V}O_{2max}$ to the normoxic ramp (solid line) and hypoxic ramp (broken line) is also provided. Error bars represent the standard error of measurement. For clarity the error bars are omitted for all but the final data point.

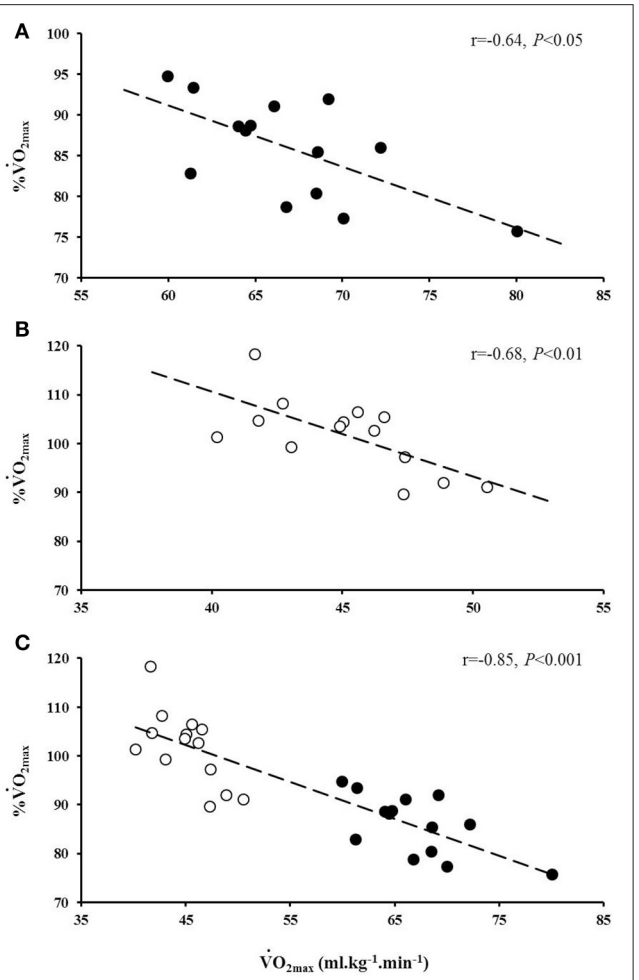

FIGURE 2 | The relationship between $\dot{V}O_{2max}$ and the percentage $\dot{V}O_{2max}$ (%$\dot{V}O_{2max}$) achieved during the normoxic (A, black circles) and hypoxic (**B**, white circles) CWR tests. The combined relationship between $\dot{V}O_{2max}$ and the % $\dot{V}O_{2max}$ achieved in normoxia and hypoxia is also provided **(C)**.

DISCUSSION

The principle novel finding of the current study was that despite being unable to attain $\dot{V}O_{2max}$ during normoxic CWR running lasting ∼2 min, highly aerobically trained individuals could achieve a hypoxia reduced $\dot{V}O_{2max}$ during CWR running of a matched duration, thus of a similar relative intensity. This is the first study to demonstrate that subjects whose $\dot{V}O_2$ plateaued below $\dot{V}O_{2max}$ during an exhaustive CWR run, were subsequently able to attain $\dot{V}O_{2max}$ when the exercise bout was replicated in hypoxic conditions despite a slowed $\dot{V}O_2$ response.

Previous research has demonstrated that during normoxic CWR running lasting ∼2 min, more highly aerobically trained individuals achieved a lower %$\dot{V}O_{2max}$ (Draper and Wood, 2005a; James et al., 2007a). In agreement with these findings, the current study reported an inverse association between $\dot{V}O_{2max}$ and the %$\dot{V}O_{2max}$ achieved during the normoxic CWR trial (**Figure 2**). To gain further insight into the relationship between $\dot{V}O_{2max}$ and %$\dot{V}O_{2max}$ achieved the present study investigated whether a hypoxia induced reduction in $\dot{V}O_{2max}$ may permit highly aerobically trained individuals to attain $\dot{V}O_{2max}$ during exhaustive CWR running at a matched relative intensity. The acute hypoxic exposure reduced $\dot{V}O_{2max}$ by ∼32%, consistent with previous reports (Engelen et al., 1996; Martin and O'Kroy, 1993; Woorons et al., 2005), and subject to this reduction $\dot{V}O_{2max}$ was achieved (**Figure 1B**). No relationship was observed between % $\dot{V}O_{2max}$ achieved and the running speed during the CWR tests in normoxia or hypoxia, nor the difference in speed between conditions (i.e., normoxia and hypoxia) and the difference in %VO_{2max} achieved (all $P > 0.05$), suggesting that $\dot{V}O_{2max}$ may be an important parameter in determining whether an individual may be able to achieve their $\dot{V}O_{2max}$ during this type of exercise. Furthermore, these findings highlight that further improvements in $\dot{V}O_{2max}$ are of less benefit to high-intensity exercise performance compared to similar gains in anaerobic capability. These findings perhaps seem incongruous with the high $\dot{V}O_{2max}$ values typically reported in elite 800 m runners (Svedenhag and Sjödin, 1984; Ingham et al., 2008) that they are apparently unable to fully utilize. However, such a high $\dot{V}O_{2max}$ value may be due to the high volume of interval training performed by these athletes (Helgerud et al.,

TABLE 1 | The parameters of the modeled $\dot{V}O_2$ response to CWR exercise in normoxia and hypoxia.

	Normoxia	Hypoxia	*P*-value
Ramp $\dot{V}O_{2max}$ (L.min^{-1})	4.40 ± 0.42	2.97 ± 0.27	<0.001
CWR $\dot{V}O_{2peak}$ (L.min^{-1})	3.79 ± 0.47	3.02 ± 0.30	<0.001
Baseline O_2 (L.min^{-1})	0.60 ± 0.11	0.67 ± 0.15	>0.05
A (L.min^{-1})	2.45 ± 0.50	1.61 ± 0.27	<0.001
Baseline + A (L.min^{-1})	3.05 ± 0.51	2.28 ± 0.21	<0.001
τ (s)	10.4 ± 2.6	12.7 ± 2.8	<0.05
δ (s)	7.6 ± 2.6	7.4 ± 3.3	>0.05

2007). There have certainly been instances where performance at altitude would indicate that the decrement in $\dot{V}O_{2max}$ may not substantially impair performance. For example, Ralph Doubell equalled the World Record at the 1968 Mexico Olympics which was performed at an altitude of 2,240 m above sea level; a feat that would seem implausible if one's $\dot{V}O_{2max}$ was a necessity for optimum performance.

The $\dot{V}O_2$ kinetics were similar to values previously reported during investigations utilizing similarly highly aerobically trained runners during CWR running lasting ∼2 min (Draper and Wood, 2005a,b; Draper et al., 2008). The phase I time delays are also similar to those reported by Wilkerson et al. (2004). Consistent with Engelen et al. (1996), we found a slower phase II τ in the hypoxic condition (**Table 1**). However, it should be noted that despite a slower phase II τ, resulting in ∼10 s difference in the attainment of the $\dot{V}O_2$ amplitude, hypoxic $\dot{V}O_{2max}$ was achieved. Conversely, despite faster $\dot{V}O_2$ kinetics normoxic $\dot{V}O_{2max}$ was not attained. Instead, there was an evident $\dot{V}O_2$ plateau in normoxia at ∼86% normoxic $\dot{V}O_{2max}$. The occurrence of a $\dot{V}O_2$ plateau, rather than a continued trajectory toward $\dot{V}O_{2max}$ and indeed the energy demands of the exercise, questions contemporary models of $\dot{V}O_2$ kinetics during CWR exercise lasting ∼2 min in this highly aerobically trained group. Interestingly, this same response is not evident during exhaustive cycle ergometry of a similar duration whereby $\dot{V}O_2$ continues to increase throughout, although maximum values are not attained (Draper et al., 2003). At present the reasons for the differences between exercise modes in $\dot{V}O_2$ response to severe intensity exercise are unclear. Increasing oxygen uptake has been associated with reduced efficiency arising from factors, such as metabolite accumulation, limitations in substrate availability, pH disturbance, increased muscle temperature, and altered motor unit recruitment (Grassi et al., 2015). Indeed, it is well established that the patterns of muscle action, including the relative proportion of eccentric and concentric contraction and the contribution of the stretch-shortening cycle differ between running and cycling (van Ingen-Schenau et al., 1997; Bijker et al., 2002). These effects might, at least in part, contribute to the between mode differences in $\dot{V}O_2$ kinetics, particularly for higher work rates where the use of elastic energy is optimized (Dalleau et al., 1998); whether or not increased stored energy during the stretch shortening cycle can help maintain efficiency despite increased metabolic fatigue warrants further investigation.

Consistent with previous investigations, we found that HR_{max} was greater in normoxia than hypoxia (Benoit et al., 1995; Mollard et al., 2007). However, similar to our $\dot{V}O_2$ findings normoxic HR_{max} was not achieved during the normoxic CWR test (Draper and Wood, 2005a,b), but hypoxic HR_{max} could be achieved during the hypoxic CWR trial. Assuming HR_{max} is needed to achieve maximal cardiac output (Q_{max}), these findings suggest that Q_{max} was not achieved during the normoxic CWR test. Despite a lower HR_{max} in hypoxia relative to normoxia, previous findings have shown that hypoxia has no effect on Q_{max} (Mollard et al., 2007), implying a compensatory increase in maximal stroke volume in hypoxia. Therefore, the inability to achieve $\dot{V}O_{2max}$ in the normoxic CWR trial may be associated with submaximal cardiac output. However, further investigation that assesses cardiac output and blood flow is necessary to gain insight into Q_{max} as a potential limiting factor in the attainment of $\dot{V}O_{2max}$ during this type of exercise.

Although end exercise $\dot{V}_E$ was greater during the CWR trials relative to the ramp incremental tests, this was not different between normoxia and hypoxia. Furthermore, we observed no differences in ventilatory equivalents between conditions (i.e., normoxia and hypoxia). These similar ventilatory responses might serve to attenuate or prevent the exercise induced arterial hypoxemia that has been described in highly aerobically trained individuals (Dempsey et al., 1984; Powers et al., 1988, 1992; Caillaud et al., 1993; review Prefaut et al., 2000). In normoxia, the increased $\dot{V}_E$ during CWR exercise would likely increase the work of breathing thereby compromising limb muscle blood flow (Wetter et al., 1999). In hypoxia, the PO_2 is in the steep portion of the oxygen-hemoglobin dissociation curve and increased $\dot{V}_E$ could have pronounced effects on arterial oxygen concentration and may help to preserve muscle $\dot{V}O_2$ despite reduced limb blood flow. However, in normoxia the PO_2 is in the flatter region of the oxygen-hemoglobin dissociation curve and the same increases in $\dot{V}_E$ would be less effective in altering arterial oxygen concentration relative to hypoxia. As a consequence the increased work associated with breathing would result in little/small increases in arterial oxygen concentration and reduce muscle blood flow and thus muscle $\dot{V}O_2$. However, it should be noted that exercise induced arterial hypoxemia has also been reported during different exercise modalities, such as cycling (Powers et al., 1988), whereas the phenomenon whereby $\dot{V}O_2$ attains a plateau below $\dot{V}O_{2max}$ has only been reported in highly aerobically trained individuals during CWR running exercise lasting ∼2 min. The mechanistic origin(s) for this phenomenon is currently unknown and requires further research.

Despite only one transition to the CWR trial in each condition, due to the large amplitude of the $\dot{V}O_2$ response during this intensity of exercise there is a much greater signal/noise ratio when compared to exercise of a lower intensity (Lamarra et al., 1987). In lesser trained individuals with smaller $\dot{V}O_2$ amplitude, thus smaller signal to noise ratio, Draper et al. (2008) demonstrated that two transitions would at worst (i.e., smallest signal to noise ratio) provide 95% confidence intervals of 1 s. Given that the current study recruited more highly aerobically trained individuals than Draper et al. (2008), thus a greater signal to noise ratio, it would be reasonable to

expect 95% confidence intervals of better than 2 s for τ. Furthermore, the current study design was sufficiently sensitive and had adequate power to detect differences in τ between conditions.

In conclusion, the results of the present study demonstrate that highly aerobically trained individuals whom are unable to achieve $\dot{V}O_{2max}$ during an exhaustive CWR run lasting ∼2 min, are able to achieve a hypoxia reduced $\dot{V}O_{2max}$ despite exhibiting slower $\dot{V}O_2$ kinetics. These data further support the notion that $\dot{V}O_{2max}$ is an important determinant of the $\%\dot{V}O_{2max}$ that can be achieved during a short duration exhaustive CWR run. The present data demonstrate that ventilatory differences are unable to explain the inability to attain $\dot{V}O_{2max}$ during normoxic CWR trials. Future research should explore the possibility of an O_2 delivery or blood perfusion limitation during this type of exercise in highly aerobically trained runners. Future research should also consider utilizing an experimental condition in normoxia which uses gradient on the treadmill (or weighted vest) instead of hypoxia to slow the running speed down and induce task failure in ∼2 min. This would aid in deciphering the novel finding of this study.

ETHICS STATEMENT

The study was approved by University of Gloucestershire Ethics Committee. All participants were provided with verbal and written information that detailed the rationale of the study, the test procedures, and any risks and benefits of participation. Participants were informed of their right to withdraw from the study at any time without penalty. All participants provided written informed consent detailing that they were willing to take part.

AUTHOR CONTRIBUTIONS

MB, CP, SD, JC, and CC were involved in conceptual design, data collection, interpretation, and manuscript preparation. All authors approve the submission of this work and agree to be accountable for all aspects of the work.

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Task Failure during Exercise to Exhaustion in Normoxia and Hypoxia Is Due to Reduced Muscle Activation Caused by Central Mechanisms While Muscle Metaboreflex Does Not Limit Performance

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To determine whether task failure during incremental exercise to exhaustion (IE) is principally due to reduced neural drive and increased metaboreflex activation eleven men (22 ± 2 years) performed a 10 s control isokinetic sprint (IS; 80 rpm) after a short warm-up. This was immediately followed by an IE in normoxia (Nx, P_IO_2:143 mmHg) and hypoxia (Hyp, P_IO_2:73 mmHg) in random order, separated by a 120 min resting period. At exhaustion, the circulation of both legs was occluded instantaneously (300 mmHg) during 10 or 60 s to impede recovery and increase metaboreflex activation. This was immediately followed by an IS with open circulation. Electromyographic recordings were obtained from the *vastus medialis* and *lateralis*. Muscle biopsies and blood gases were obtained in separate experiments. During the last 10 s of the IE, pulmonary ventilation, VO_2, power output and muscle activation were lower in hypoxia than in normoxia, while pedaling rate was similar. Compared to the control sprint, performance (IS-Wpeak) was reduced to a greater extent after the IE-Nx (11% lower $P < 0.05$) than IE-Hyp. The root mean square (EMG_{RMS}) was reduced by 38 and 27% during IS performed after IE-Nx and IE-Hyp, respectively (Nx vs. Hyp: $P < 0.05$). Post-ischemia IS-EMG_{RMS} values were higher than during the last 10 s of IE. Sprint exercise mean (IS-MPF) and median (IS-MdPF) power frequencies, and burst duration, were more reduced after IE-Nx than IE-Hyp ($P < 0.05$). Despite increased muscle lactate accumulation, acidification, and metaboreflex activation from 10 to 60 s of ischemia, IS-Wmean (+23%) and burst duration (+10%) increased, while IS-EMG_{RMS} decreased (−24%, $P < 0.05$), with IS-MPF and IS-MdPF remaining unchanged. In conclusion, close to task failure, muscle activation is lower in hypoxia than in normoxia. Task failure is predominantly caused by central mechanisms, which recover to great extent within 1 min even when the legs remain ischemic. There is dissociation between the recovery of EMG_{RMS} and performance. The reduction of surface electromyogram MPF, MdPF and burst duration due to fatigue is

associated but not caused by muscle acidification and lactate accumulation. Despite metaboreflex stimulation, muscle activation and power output recovers partly in ischemia indicating that metaboreflex activation has a minor impact on sprint performance.

Keywords: electromyography, EMG, exhaustion, fatigue, high-intensity, hypoxia, lactate, performance

INTRODUCTION

Muscle fatigue has been defined as "any exercise-induced reduction in the ability to exert muscle force or power, regardless of whether the task can be sustained (Bigland-Ritchie and Woods, 1984), that can be reversed by rest" (Gandevia, 2001). The mechanisms leading to task failure may involve physiological processes at neural (central fatigue) or muscular levels (peripheral fatigue), with failure distal to the neuromuscular junction included in the "peripheral" component. It has been suggested that the rate of muscle fatigue development is regulated by the central nervous system (CNS) with feedback from the type III and IV muscle afferents (Amann and Dempsey, 2008), which sense metabolite accumulation, particularly H^+, lactate, and ATP (Light et al., 2008). Type III/IV muscle afferents have been reported to inhibit corticospinal drive (Amann and Dempsey, 2008; Rossman et al., 2012; Kennedy et al., 2015), with greater inhibitory effect on extensor than flexor muscles (Martin et al., 2006). However, whether metabolite accumulation and the expected metaboreflex stimulation impair muscle activation and limit peak power during whole-body sprint exercise remains unknown.

During whole-body exercise in severe acute hypoxia ($F_IO_2 < 0.115$, or altitude above 4500 m) the level of peripheral fatigue at exhaustion seems lower, indicating that central mechanisms, likely linked to reduced brain oxygenation (Rasmussen et al., 2010), predominate over local mechanisms in determining the cessation of exercise (Amann et al., 2007). In agreement with this hypothesis, an instantaneous increase of the inspired O_2 fraction (F_IO_2) from 0.21 to 1.00 does not eliminate muscle fatigue at the end of an incremental exercise test performed at sea level (Calbet et al., 2003a). In contrast, during constant-intensity or incremental exercise to exhaustion in severe hypoxia ($P_IO_2 \approx 75$ mmHg), muscle fatigue is swiftly relieved by mild hyperoxic gas or room air (Kayser et al., 1994; Calbet et al., 2003a,b; Amann et al., 2007). These findings led to the concept that during incremental exercise to exhaustion in normoxia, task failure is most likely caused by peripheral mechanisms while central mechanisms prevail in severe hypoxia (Calbet et al., 2003a; Amann et al., 2007). In support, peripheral fatigue, as assessed via decreases in potentiated quadriceps twitch force 2 min after constant-intensity exercise to exhaustion, was lower when the exercise was performed in severe hypoxia than in normoxia (Amann et al., 2007). Nevertheless, this observation was not accompanied by an assessment of muscle metabolites and obviates the fact that reduced potentiated quadriceps twitch force may occur without reduction of peak power output (Fernandez-del-Olmo et al., 2013; Hureau et al., 2014). Moreover, the recovery process starts as early as muscle contraction ceases, and given the fast kinetics of phosphocreatine re-synthesis at the end of exercise (Bogdanis et al., 1996; Dawson et al., 1997; Yoshida et al., 2013), most of the recovery has already occurred within the first 2 min post-exercise (Sargeant and Dolan, 1987; Froyd et al., 2013). Also, muscle fatigue is task-specific (Gandevia, 2001), implying that a procedure using a similar pattern of movement, and hence recruitment of neural pathways, is expected to be more sensitive to detect fatigue.

In this context, every possible combination of effects and interpretation of results has been reported in regard to the contribution of central and peripheral mechanisms to muscle fatigue after dynamic contractions in normoxia (Sidhu et al., 2009, 2012; Marcora and Staiano, 2010; Fernandez-del-Olmo et al., 2013) and hypoxia (Amann et al., 2007; Goodall et al., 2010; Millet et al., 2012). Some of these discrepancies can be attributed to the fact that neural mechanisms of muscle fatigue are task-specific (Sidhu et al., 2012), to different levels of input from type III and IV muscle afferents (Sidhu et al., 2012), and to methodological limitations (Rodriguez-Falces et al., 2013; Héroux et al., 2015; Bachasson et al., 2016). Although the inhibition of type III and IV muscle afferents has been shown to attenuate muscle fatigue in certain exercise models (Sidhu et al., 2014), whether type III and IV muscle afferent input contributes to reduce exercise performance by a central mechanism remains controversial (Millet et al., 2009, 2012; Marcora, 2010; Amann et al., 2013a; Kennedy et al., 2015). Part of the discrepancies may be due to the fact that type III and IV muscle afferent discharge cannot be directly measured during whole-body exercise in humans, combined with the difficulty in interpreting the effects of intrathecal fentanyl when this drug differently alters ventilation, arterial O_2 content, arterial $PaCO_2$, heart rate and mean arterial blood pressure, depending on the exercise intensity, exercise duration, and the study population (Dempsey et al., 2014; Olson et al., 2014; Poon and Song, 2015). Thus, we decided to explore the role of III/IV muscle afferents on exercise performance (peak power output) using a completely different experimental approach.

Abbreviations: ADP, Adenosine diphosphate; ATP, Adenosine triphosphate; CNS, Central nervous system; d.w., Dry weight; DEXA, Dual-energy x-ray absorptiometry; EMG, Surface electromyogram; EMG_{RMS}, Root mean square of the EMG; F_IO_2, Inspired oxygen fraction; HR, Heart rate; HRmax, Maximal heart rate; Hyp, Hypoxia; Hypb, session in hypoxia with biopsies taken; IE, Incremental exercise to exhaustion; IS, Isokinetic sprint; MPF, Mean power frequency; MdPF, Median power frequency; MVC, Maximal voluntary contraction; Nx, Normoxia; Nxb, Session in normoxia with biopsies taken; PaO_2, Arterial oxygen pressure; PCr, Phosphocreatine; P_IO_2, Inspired O_2 pressure; RMS, root mean square; RMSNz, Normalized root mean square; V_E, Minute ventilation; VO_2, Oxygen consumption; VO_2max, Maximal oxygen uptake; VO_2peak, Peak oxygen uptake; Wpeak-i, Instantaneous peak power output; Wmax, Peak power output at exhaustion during the incremental exercise test; Wmean, mean power output during the 10 s sprints; w.w., wet weight; TAI, Total activation index.

The main aim of this investigation was to determine whether task failure during an incremental exercise to exhaustion is principally due to central mechanisms that cause a reduction in neural activation, modulated by the level of oxygenation. This hypothesis has been previously examined but with different procedures (Kayser et al., 1994; Amann et al., 2007, 2013b) which did not include measurements of muscle metabolites or performance immediately after exhaustion. Another aim was to determine if increased afferent feedback from metabolite accumulation in an exhausted muscle has a negative influence on sprint performance by reducing neural activation, as assessed through electromyogram (EMG) recordings.

We aimed to test these two hypotheses: (i) task failure during incremental exercise to exhaustion in hypoxia occurs with lower levels of muscle activation compared to normoxia, and (ii) increased afferent feedback from III and IV muscle afferents impairs sprint performance.

METHODS

Subjects

Eleven healthy men (age: 21.5 ± 2.0 years, height: 174 ± 8 cm, body mass: 72.3 ± 9.3 kg, body fat: 16.1 ± 4.9%, VO_2max: 51 ± 5 mL.kg^{-1}.min^{-1}) agreed to participate in this investigation. Before volunteering, subjects received full oral and written information about the experiments and possible risks associated with participation. Written consent was obtained from each subject. The study was performed by the Helsinki Declaration and was approved by the Ethical Committee of the University of Las Palmas de Gran Canaria (CEIH-2010-01 and CEIH-2009-01).

General Overview

This study was a part of a larger project that included several experiments designed to address the mechanisms limiting whole-body exercise performance in humans. The results focusing on O_2 transport and muscle metabolism have been published (Calbet et al., 2015; Morales-Alamo et al., 2015). Body composition was determined by dual-energy x-ray absorptiometry (DEXA) (Hologic QDR-1500, Hologic Corp., software version 7.10, Waltham, MA) (Calbet et al., 1997), during the familiarization sessions. The leg muscle mass was calculated from the DEXA scans using Wang's et al. model (Wang et al., 1999).

The experimental protocol is summarized in **Figure 1**, and an example of the electromyographic recordings from one subject is given in **Figure 2**. On the experimental days, subjects reported to the laboratory at 08.00 h. after an overnight fast from 22.00 h. The subjects performed an incremental exercise test to exhaustion in normoxia (P_IO_2: ∼143 mmHg) or acute hypoxia (P_IO_2: ∼73 mmHg, Altitrainer200, SMTEC, Switzerland), in random order and separated by a 120 min rest. Before the exercise test, bilateral cuffs were placed around the thighs and connected to a rapid cuff inflator (SCD10, Hokanson E20 AG101, Bellevue, USA). The test started with a warm-up (2 min at 50 W + 2 min 100 W + 1 min at 160 W) followed by 4.5 min of slow unloaded pedaling. This was followed by a 30 s rest period while the subjects became ready to sprint at the 5th minute after the end of the warm-up. The volunteers were requested to sprint as hard and fast as possible during 10 s with the ergometer set in isokinetic mode and at 80 rpm (Excalibur Sport 925900, Lode, Groningen, The Netherlands). This sprint was used as a control sprint and was always performed in normoxia. Five minutes later, the incremental exercise began. For the test in normoxia, the load was increased by 30 W every 2 min until exhaustion, starting from an initial load of 80 W. In hypoxia, the incremental test started from 60 W, and the load was increased by 20 W every 2 min until exhaustion. Exhaustion during the incremental exercise tests was defined by the subject stopping pedaling or dropping pedaling rate below 50 rpm during 5 s, despite strong verbal encouragement. At exhaustion, the cuffs were inflated at maximal speed and pressure (i.e., 300 mmHg) to completely and instantaneously occlude the circulation (ischemia). This prevented any increase of oxygenation during the recovery and caused anoxia within 3–5 s of the application of the occlusion as reported elsewhere (Morales-Alamo et al., 2015). A limitation of previous studies was that the impact of the early recovery could not be accounted for (Marcora and Staiano, 2010; Coelho et al., 2015), and certainly some recovery occurs during the time elapsed between the end of the exercise and the start of the sprint. To circumvent this limitation we applied complete ischemia during the recovery and we used short (10 s) and long (60 s) ischemia periods. We surmised that peripheral fatigue would be exacerbated by the prolonged ischemia at the end of an incremental exercise to exhaustion.

The incremental exercise test in normoxia and hypoxia ended with two different periods of ischemia of 10 or 60 s, during which the subjects breathed normoxic gas. Following a countdown, the subject performed a 10 s isokinetic sprint as hard and fast as possible while the ergometer was set at 80 rpm. The cuffs were always instantaneously deflated at the beginning of the post-ischemia sprints. In the unfatigued state, peak power increases with pedaling rate, but peak power is less affected by pedaling rate in the fatigued state (Beelen and Sargeant, 1991). Importantly, the difference in peak power between the fatigued and unfatigued state increases the higher the pedaling rate used in the control sprint (unfatigued) (Beelen and Sargeant, 1991). Thus, to avoid the limitations associated with varying pedaling rates sprints were performed in isokinetic mode at 80 rpm, a pedaling rate that allows maximal power output in the fatigued state (Beelen and Sargeant, 1991).

A few weeks later, the IEs were repeated in two additional experimental sessions; 1 day in normoxia (Nxb, P_IO_2: ∼143 mmHg) and the other in hypoxia (Hypb, P_IO_2: ∼73 mmHg; "b" indicates biopsy session). In the Nxb session, after 10 min rest in the supine position, a muscle biopsy was obtained from the m. *vastus lateralis* with local anesthesia (lidocaine 2%, 2 ml), using the Bergstrom technique with suction (Bergstrom, 1962). This biopsy was obtained with the needle pointing distally with 45° inclination (Guerra et al., 2011). An additional incision was performed before the beginning of the exercise in the contralateral leg. Afterward, the incisions were covered with a transient plaster, and a cuff was placed around the left leg. The subjects then sat on the cycle ergometer and resting

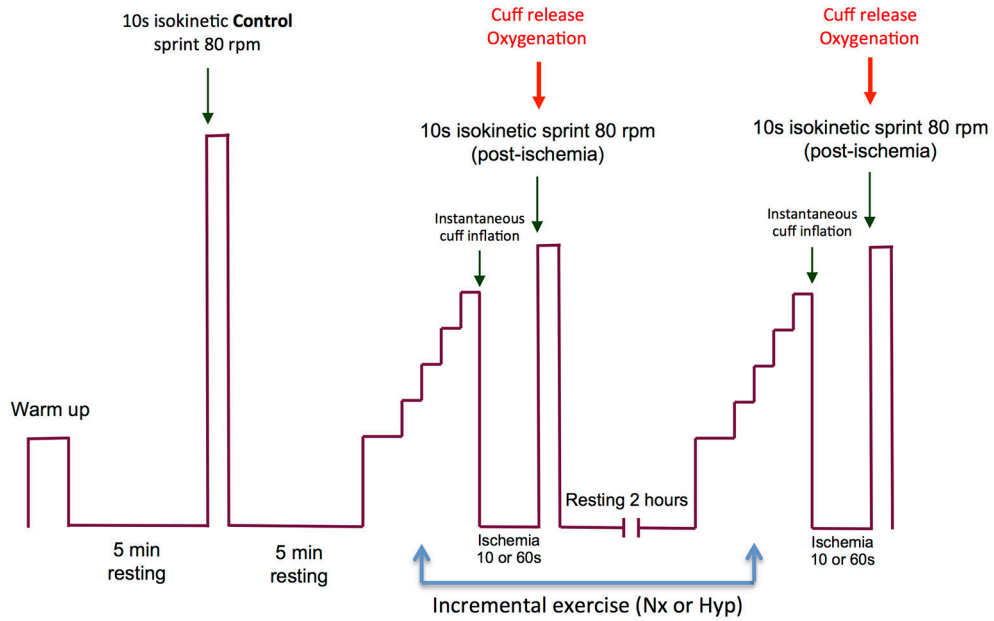

FIGURE 1 | Experimental protocol. The experimental day started with a warm-up followed by 4.5 min of slow unloaded pedaling and a 30 s resting phase, while the subjects became ready to perform the first sprint (isokinetic, 10 s at 80 rpm) at the 5th minute after the end of the warm-up. This sprint was used as a control sprint and was always performed in normoxia. Five minutes later, an incremental exercise to exhaustion began in normoxia (P_IO_2: ~143 mmHg) or acute hypoxia (P_IO_2: ~73 mmHg). The order of the incremental exercise test was randomized. Between the two incremental exercise tests, the subjects were allowed to rest during 120 min. At the end of the incremental exercise test, bilateral cuffs were inflated at maximal speed and pressure (i.e., 300 mmHg) to occlude completely and instantaneously the circulation (ischemia) of the legs. The incremental exercise test in normoxia and hypoxia ended with an ischemia period of 10 s on one experimental day and 60 s on another day. The order of the duration of the ischemia period was randomized. At the end of the ischemia period, the subjects performed a 10 s isokinetic sprint as hard and fast as possible (80 rpm) while the cuffs were always instantaneously deflated at the beginning of the post-ischemia sprints.

measurements were performed. Two minutes later, the IE was begun as described above. At exhaustion, the cuff was inflated instantaneously at 300 mmHg, and a biopsy was taken exactly 10 s after the end of the incremental exercise test. The biopsy needle was introduced perpendicular to the thigh. This biopsy was followed by a final biopsy at 60 s with the needle pointing proximally (45° inclination). In the Hypb session, essentially the same procedures were applied. All biopsies were immediately frozen in liquid nitrogen and stored at −80°C. Hypb and Nxb sessions were performed in random order.

Power Output, Oxygen Uptake and Hemoglobin Oxygen Saturation

Power output during the sprint was reported as instantaneous peak power output (Wpeak-i) and mean power output (Wmean) during the 10 s duration of the sprint. Oxygen uptake was measured with a metabolic cart (Vmax N29; Sensormedics, Yorba Linda, California, USA), calibrated before each test according to the manufacturer instructions, with high-grade calibration gasses (Carburos Metálicos, Las Palmas de Gran Canaria, Spain). Respiratory variables were analyzed breath-by-breath and averaged every 20 s during the incremental exercise tests. The highest 20-s averaged VO_2 recorded in normoxia was taken as the VO_2max. The same criterion was applied to determine the VO_2peak in hypoxia. Hemoglobin oxygen saturation (SpO_2) was determined with a finger pulse oximeter (OEM III module, 4549-000, Plymouth, MN).

Muscle Metabolites

From each muscle biopsy, 30 mg of wet tissue were freeze-dried, cleaned and powdered with a manual mortar on ice. Subsequently, the samples were suspended in 0.5 M $HClO_4$ and centrifuged at 15,000 g at 4°C for 15 min. The supernatant was neutralized with $KHCO_3$ 2.1M. ATP, phosphocreatine (PCr), creatine, pyruvate and lactate concentrations were enzymatically determined in neutralized extracts by fluorometric analysis (Lowry and Passonneau, 1972; Morales-Alamo et al., 2013).

Electromyography

Electrical muscle activation was monitored using surface electromyography (EMG). EMG signals were continuously recorded from the *vastus medialis* and *vastus lateralis*. Before the application of the EMG electrodes the skin surface was carefully shaved and wiped with alcohol to reduce skin impedance. Bipolar single differential electrodes were placed longitudinally on the muscles following the SENIAM recommendations (Merletti and Hermens, 2000) and taped to the skin to minimize movement artifacts. The reference electrode was placed on the skin over the acromion. The position of the electrodes was marked on the skin with indelible ink, and these references were used for precise electrode placement on repeated experiments.

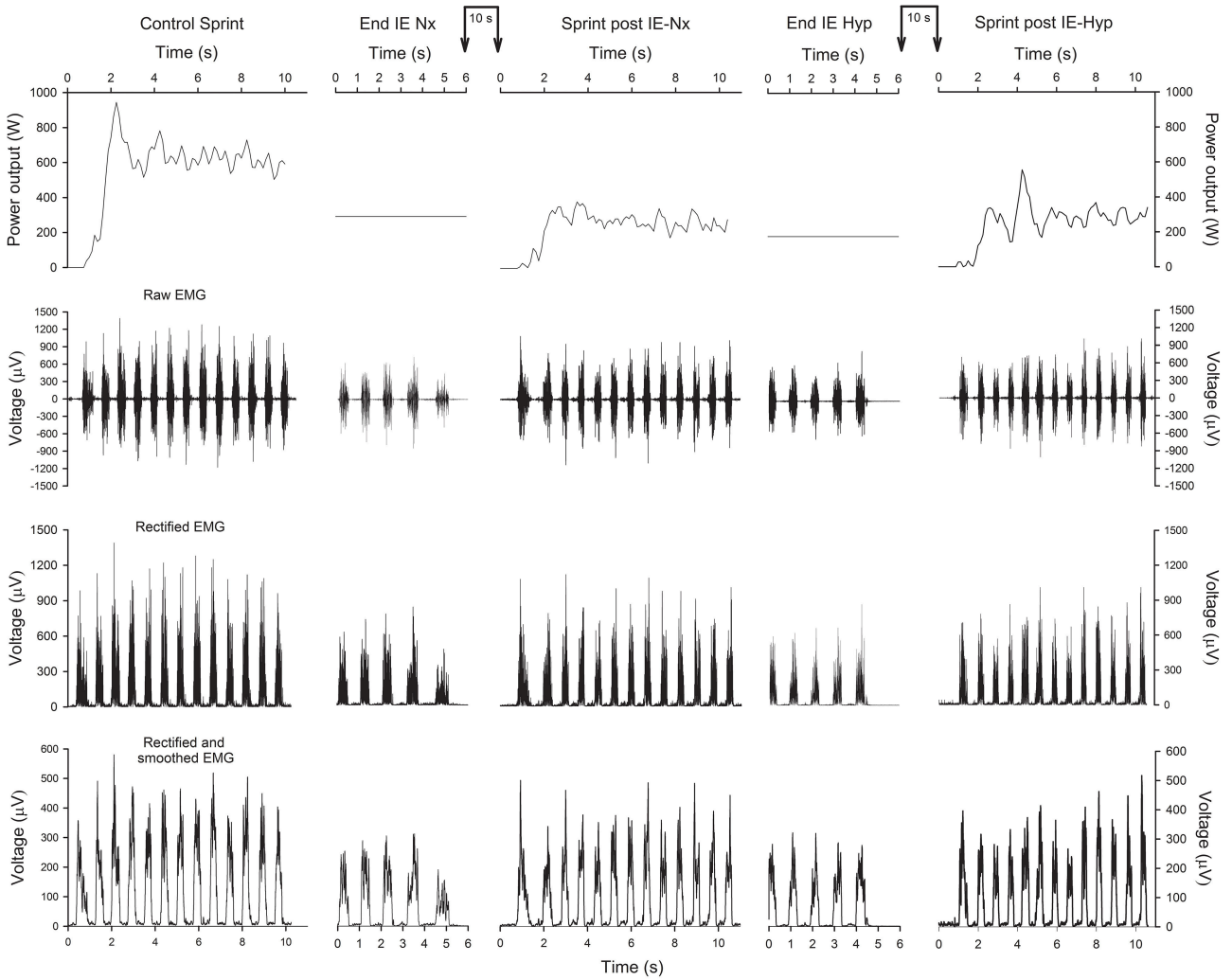

FIGURE 2 | Power output and EMG of a representative subject. Schematic representation of the power output (upper panels), raw EMG (2nd row), rectified EMG (3rd row) and rectified and smoothed EMG (lower panels), during the control sprint, last 6 s of the incremental exercise (IE) in normoxia (Nx), subsequent 10-s isokinetic sprint at 80 rpm (normoxia), IE in hypoxia (Hyp) and subsequent 10-s isokinetic sprint at 80 rpm (normoxia). The connected vertical arrows indicate the duration of the ischemia period, which in this example was 10 s.

The EMG signals were acquired using a 16-channel recording system (Myomonitor IV, Delsys Inc., Boston, MA) at a sampling rate of 1000 Hz using rectangular shaped (19.8 mm wide and 35 mm long) bipolar surface electrodes with 1 × 10 mm 99.9% Ag conductors, and with an inter-conductor distance of 10 mm (DE-2.3 Delsys Inc.). The EMG data were filtered with a high-pass filter of 20 Hz and a low-pass filter of 450 Hz using a fifth-order Butterworth filter. The system has an input impedance of $> 10^{15}\Omega$ per 0.2pF of input capacitance, a common mode rejection ratio of >80 dB, signal-to-noise ratio <1.2 μV, and a pre-amplifier gain 1000 V/V ±1%. Each pedal revolution was detected using an electrogoniometer (Goniometer Biosignal Sensor S700 Joint Angle Shape Sensor; Delsys Inc. Boston) fixed on the left knee and sampled at 500 Hz. EMG and joint movement were simultaneously recorded by a portable device (Myomonitor IV, Delsys Inc. Boston) and wirelessly transmitted to a computer (EMGWorks Wireless application and EMGWorks Acquisition 3.7.1.3; Delsys, Inc. Boston).

The EMG signal corresponding to each muscle contraction was analyzed using code developed "in house" (Matlab R2012b, MathWorks, Natick, MA, USA). The EMG recordings were full-wave rectified and to provide an index of muscle activation, the amplitude characteristics were analyzed via average RMS of a 25-ms moving window for the duration of the burst. Burst onset and offset detection were determined using 20% of the maximal EMG_{RMS} activity of each burst as a reference (Baum and Li, 2003; Hug and Dorel, 2009; Torres-Peralta et al., 2014), rather than a mean threshold value from 15 consecutive bursts (Ozgünen et al., 2010). This approach yielded the same result as direct simple visual discrimination with 100% detection of all

bursts. The EMG_{RMS} recorded during the last minute of a 2 min 80 W load (in normoxia) was used to normalize the remaining EMG_{RMS} data. Besides, we defined a total activity index during the sprint (TAI) as TAI = EMG_{RMS} × burst duration (ms) × number of pedal strokes during the sprint. The total activity index is similar to the integrated EMG signal, but was computed separately for each burst and excluded the baseline EMG between burst (Torres-Peralta et al., 2014). The TAI recorded during the last minute of a 2 min 80 W load (in normoxia) was used to normalize the rest of the TAI values.

The mean (MPF) and median (MdPF) power spectrum frequencies were calculated using the Fast Fourier Transform (Solomonow et al., 1990). All variables were reported as the mean values of the pedal strokes recorded during the last 10 s of the incremental exercise or the 10 s sprints. EMG variables responded similarly during the four control sprints. Therefore, to reduce EMG variability the four control sprints were averaged. EMG data are reported separately for *vastus medialis* and *lateralis*, and also as the average of the two muscles. Since the incremental exercise tests in normoxia and hypoxia were repeated, the mean of each pair was used in further analysis to represent either normoxia or hypoxia.

Statistics

Normal distribution of variables was checked with the Shapiro-Wilks test. A repeated-measures ANOVA with F_IO_2 condition (normoxia vs. hypoxia) and occlusion duration (10 vs. 60 s) was used to analyze the responses observed during the sprints. Pairwise comparisons at specific time points were performed with Student t-tests, and adjusted for multiple comparisons using the Holm–Bonferroni method. Values are reported as the mean ± standard deviation (unless otherwise stated). $P \leq 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS v.15.0 for Windows (SPSS Inc., Chicago, IL) and Excel 2011 (Microsoft, Redmond, WA, USA).

RESULTS

Incremental Exercise

Compared to normoxia, Wmax, pulmonary ventilation, respiratory rate, heart rate and VO_2peak were reduced during the last 10 s of exercise in hypoxia. Additional information regarding the respiratory and cardiovascular responses to the IE can be found elsewhere (Calbet et al., 2015; Morales-Alamo et al., 2015). The ergospirometric variables corresponding to the last 10 s of incremental exercise are reported in **Table 1**, together with the corresponding electromyographic responses.

Muscle Fatigue

Compared to the control sprints, post-IE sprint performance was reduced (32–46%, $P < 0.05$; **Figure 3**) as previously reported (Morales-Alamo et al., 2015). Sprint Wpeak-i was 11% lower following post-IE in normoxia than hypoxia. Similar effects were observed in Wmean. Sprint performance was improved after ischemic recovery. Wpeak-i and Wmean were 11 and 23% higher, respectively, following 60 than 10 s of occlusion ($P < 0.05$), as previously reported (Morales-Alamo et al., 2015).

TABLE 1 | Ergospirometric and electromyographic responses during the last 10 s of the incremental exercise to exhaustion in normoxia (P_IO_2 ≈ 143 mmHg) and severe hypoxia (P_IO_2 ≈ 73 mmHg).

	Normoxia	Hypoxia	*P*
F_IO_2 (%)	20.8 ± 0.1	10.8 ± 0.1	<0.001
SpO_2(%)	93.1 ± 4.4	64.6 ± 4.6	<0.001
Wmax (W)	259.2 ± 32.0	170.1 ± 21.0	<0.001
VO_2peak (l.min^{-1})	3.62 ± 0.37	2.41 ± 0.29	<0.001
V_E (l.min^{-1})	143.5 ± 19.5	123.8 ± 22.5	<0.001
RR (breaths.min^{-1})	60.2 ± 7.6	53.8 ± 7.0	<0.001
HR (beats.min^{-1})	185.4 ± 6.0	175.1 ± 9.0	<0.001
$P_{ET}O_2$(mmHg)	113.2 ± 2.3	58.6 ± 2.8	<0.001
$P_{ET}CO_2$(mmHg)	32.1 ± 2.6	27.5 ± 2.1	<0.001
RER	1.15 ± 0.03	1.35 ± 0.11	<0.001
VCO_2(l.min^{-1})	4.12 ± 0.49	3.23 ± 0.41	<0.001
Pedaling rate (rpm)	56.7 ± 7.4	55.4 ± 5.9	0.5
VM RMSraw (μV)	134.2 ± 60.1	113.3 ± 48.9	<0.05
VL RMSraw (μV)	103.0 ± 30.9	88.9 ± 30.9	0.12
Average RMSraw (μV)	121.0 ± 37.7	101.1 ± 33.7	<0.01
VM RMSNz (A.U.)	219.1 ± 77.6	182.3 ± 45.5	<0.05
VL RMSNz (A.U.)	193.7 ± 76.4	165.0 ± 62.4	0.07
Average RMSNz (A.U.)	206.4 ± 61.2	173.7 ± 41.8	<0.05
VM TAINz (A.U.)	289.5 ± 109.6	222.6 ± 88.1	<0.001
VL TAINz (A.U.)	246.5 ± 102.2	187.5 ± 73.1	<0.05
Average TAINz (A.U.)	268.0 ± 80.1	205.0 ± 68.2	<0.001
VM MPF (Hz)	84.2 ± 18.4	89.6 ± 18.0	0.13
VL MPF (Hz)	84.1 ± 18.6	89.6 ± 17.8	0.13
Average MPF (Hz)	84.2 ± 18.5	89.6 ± 17.9	0.13
VM MdPF (Hz)	68.6 ± 12.3	73.3 ± 14.8	<0.05
VL MdPF (Hz)	68.5 ± 12.5	73.0 ± 14.6	<0.05
Average MdPF (Hz)	68.6 ± 12.4	73.1 ± 14.7	<0.05
VM Burst (ms)	401.9 ± 51.7	362.5 ± 55.9	0.12
VL Burst (ms)	401.4 ± 55.2	368.3 ± 55.7	0.10
Average Burst (ms)	401.7 ± 49.4	365.4 ± 52.8	0.10

F_IO_2, inspiratory oxygen fraction; SpO_2, hemoglobin saturation in capillary blood measured by pulse oximetry; Wmax, power output at exhaustion; VO_2, oxygen consumption; V_E, pulmonary ventilation; RR, respiratory rate; HR, heart rate; $P_{ET}O_2$, end-tidal O_2 pressure; $P_{ET}CO_2$, end-tidal CO_2 pressure; RER, respiratory exchange ratio; VCO_2, CO_2 production; rpm, revolutions per minute; VL, vastus lateralis; VM, vastus medialis; RMSraw, raw root mean square; RMSNz, normalized root mean square; TAINz, Normalized total activation index (arbitrary units, A.U.); MPF, mean power frequency; MdPF, median power frequency; Burst, burst duration; Average, mean of VM and VL.

EMG responses

Vastus Lateralis EMGs

Figure 2 depicts an example of the EMG recordings during one experimental day. During the last 10 s of the incremental exercise to exhaustion the raw (average of the two muscles) and normalized RMS were 16% lower in hypoxia than in normoxia ($P < 0.05$) (**Table 1**). The average normalized TAI was also lower (23%) in hypoxia than in normoxia ($P < 0.001$). The median frequency was 6% lower in normoxia than hypoxia ($P < 0.05$). The mean and median frequencies during the last 10 s of the IE remained at the same level during the subsequent sprints.

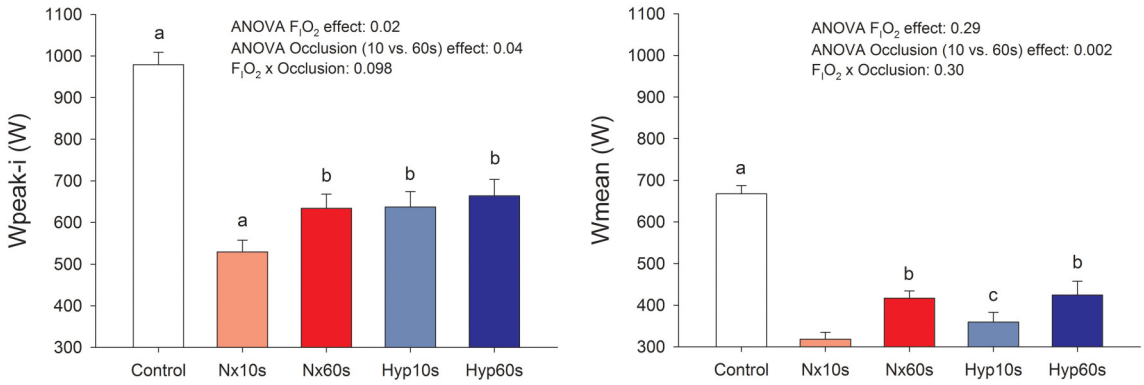

FIGURE 3 | Power output. Peak (Wpeak-i) and mean (Wmean) power output during sprint exercise performed at the end of an incremental exercise to exhaustion in normoxia ($P_IO_2 \approx 143$ mmHg) and severe hypoxia ($P_IO_2 \approx 73$ mmHg), after 10 or 60 s of occlusion of the circulation. $^aP < 0.05$ compared with the other conditions; $^bP < 0.05$ compared with Nx10s; $^cP < 0.05$ compared with Nx60s.

TABLE 2 | *Vastus lateralis* electromyographic variables in response to sprint exercise performed at the end of an incremental exercise to exhaustion in normoxia ($P_IO_2 \approx 143$ mmHg) and severe hypoxia ($P_IO_2 \approx 73$ mmHg), after 10 or 60 s of occlusion of the circulation.

	Control sprint	Nx10s	Nx60s	Hyp10s	Hyp60s	Main Oxy (*P*)	Main Occ (*P*)	Interaction Oxy × Occ (*P*)
RMSraw (μV)	238.1 ± 82.0^a	153.2 ± 64.7	137.1 ± 53.2	160.2 ± 68.7	161.8 ± 61.8	0.220	0.706	0.429
RMSNz (A.U.)	475.1 ± 155.2^a	317.3 ± 95.1	254.6 ± 110.0	344.2 ± 126.4^c	317.0 ± 172.4	0.112	0.059	0.436
Burst (ms)	$331.9 \pm 24.6^{b,d}$	299.7 ± 22.7^c	324.6 ± 27.7	305.5 ± 23.8^c	$337.3 \pm 34.4^{b,d}$	0.026	0.001	0.381
TAINz (A.U.)	1023.1 ± 340.6^a	600.8 ± 268.8	581.5 ± 244.2	642.9 ± 297.0	715.8 ± 288.2	0.12	0.729	0.326
MPF (Hz)	$93.9 \pm 9.7^{b,c}$	81.9 ± 14.2	79.5 ± 16.4	86.3 ± 20.3	89.5 ± 17.2	0.006	0.837	0.212
MdPF (Hz)	$80.2 \pm 6.0^{b,c,d}$	66.4 ± 9.4^e	67.0 ± 13.6	71.4 ± 16.6	74.7 ± 13.9^c	0.011	0.255	0.500

$^aP < 0.05$ compared with the other conditions.
$^bP < 0.05$ compared with Nx10s.
$^cP < 0.05$ compared with Nx60s.
$^dP < 0.05$ compared with Hyp10s.
$^eP < 0.05$ compared with Hyp60s.
RM ANOVA (2 × 2) Main Oxy: main oxygenation effect due to the conditions in which was performed the incremental exercise test (Nx, Normoxia; Hyp, hypoxia); RM ANOVA (2 × 2) Main Occ, Main Occlusion effect due to the duration of the occlusion (10 vs. 60 s); Wpeak-i, instantaneous peak power in the sprint; Wmean, mean power in the 10 s sprint; RMSraw, raw root mean square; RMSNz, Normalized root mean square; Burst, burst duration; TAINz, Normalized total activation index (arbitrary units, A.U.); MPF, mean power frequency; MdPF, median power frequency.

The average RMS, RMSNz, and TAINz were 1.6-, 1.7-, and 2.9-fold higher during the sprints post-IE than during the last 10 s of the IE (all, $P < 0.05$). Compared to the control sprints, the RMSraw and the normalized RMSNz were reduced by 36 and 35%, respectively, in the sprints performed after the IE (**Table 2**). Although the 60 s occlusion caused a 14% lower RMSNz compared to the 10 s occlusion, this difference did not reach statistical significance (Main effect $P = 0.059$). Compared to the control sprints the TAINz was reduced by 42 and 34% during the sprints performed after the incremental test in normoxia and hypoxia, respectively ($P < 0.05$).

Compared to the control sprint, the median frequency was reduced by 18 and 9% in the sprints that followed the IEs in normoxia and hypoxia, respectively (both, $P < 0.05$) (**Table 3**). Thus, the median frequency was 9% lower in the sprints that followed IE in normoxia than in hypoxia (Main effect $P = 0.011$). MPF changes were essentially similar to MdPF (**Tables 1–4**).

Compared to the control sprints, the duration of the bursts was reduced by 9% in the sprints performed after 10 s occlusions. Nevertheless, after 60 s of occlusion, the duration of burst in the following sprints achieved the same value as in the control sprints (331.9 ± 24.6 and 331.0 ± 29.7 ms, control and 60 s after IE, respectively, $P = 0.91$). The duration of the bursts was 3% longer in the sprints that followed an IE in hypoxia than normoxia ($P = 0.03$). The average contraction time (i.e., burst duration x pedaling rate) was 15% lower during the last 10 s of IE than during the post-IE sprints ($P < 0.001$).

Vastus Medialis EMGs

The *vastus medialis* EMG responses to incremental (**Table 1**) and sprint exercise (**Table 3**) were similar to those described for the *vastus lateralis*. Therefore, we combined both *vastus* EMGs responses to reduce variability (**Table 4**). The combined response was similar to that reported for *vastus lateralis* and *medialis*, but with lower variability, confirming some of the effects that did not reach statistical significance when only analyzed using a single muscle EMG recordings.

TABLE 3 | *Vastus medialis* electromyographic variables in response to sprint exercise performed at the end of an incremental exercise to exhaustion in normoxia and severe hypoxia (P_IO_2 ≈ 73 mmHg), after 10 or 60 s of occlusion of the circulation.

	Control sprint	Nx10s	Nx60s	Hyp10s	Hyp60s	Main Oxy (*P*)	Main Occ (*P*)	Interaction Oxy × Occ (*P*)
RMSraw (μV)	263.5 ± 115.7[b,c,e]	177.4 ± 95.0[d]	193.9 ± 121.3	204.2 ± 103.1	183.9 ± 81.1	0.333	0.943	0.038
RMSNz (A.U.)	410.4 ± 163.3[a]	284.0 ± 97.1[d]	276.4 ± 126.1	333.0 ± 143.1	279.7 ± 104.8[d]	0.078	0.137	0.098
Burst (ms)	342.2 ± 27.7[b]	309.4 ± 42.5[c]	355.2 ± 49.4[d]	322.0 ± 53.1	347.8 ± 37.9[b]	0.743	0.017	0.154
TAINz (A.U.)	1152.1 ± 463.3[a]	701.1 ± 359.9[d]	809.0 ± 498.6	829.7 ± 397.9	820.7 ± 335.6	0.118	0.638	0.119
MPF (Hz)	94.3 ± 9.9[b,c,d]	81.9 ± 14.0	79.8 ± 15.8	85.8 ± 19.2	89.4 ± 17.4	0.008	0.703	0.241
MdPF (Hz)	80.8 ± 6.7[a]	66.2 ± 9.2	67.1 ± 13.4	71.3 ± 16.6	74.6 ± 14.1[b,c]	0.011	0.218	0.587

[a] *P < 0.05 compared with the other conditions.*
[b] *P < 0.05 compared with Nx10s.*
[c] *P < 0.05 compared with Nx60s.*
[d] *P < 0.05 compared with Hyp10s.*
[e] *P < 0.05 compared with Hyp60s.*
RM ANOVA (2 × 2) Main Oxy, main oxygenation effect due to the conditions in which was performed the incremental exercise test (Nx, Normoxia; Hyp, hypoxia); RM ANOVA (2 × 2) Main Occ, Main Occlusion effect due to the duration of the occlusion (10 vs. 60 s); RMSraw, raw root mean square; RMSNz, Normalized root mean square; Burst, burst duration; TAINz, Normalized total activation index (arbitrary units, A.U.); MPF, mean power frequency; MdPF, median power frequency.

TABLE 4 | Electromyographic variables (average of *vastus medialis* and *lateralis*) in response to sprint exercise performed at the end of an incremental exercise to exhaustion in normoxia (P_IO_2 ≈ 143 mmHg) and severe hypoxia (P_IO_2 ≈ 73 mmHg), after 10 or 60 s of occlusion of the circulation.

	Control	Nx10s	Nx60s	Hyp10s	Hyp60s	Main Oxy (*P*)	Main Occ (*P*)	Interaction Oxy × Occ (*P*)
RMSraw (μV)	250.8 ± 89.1[a]	165.3 ± 69.6[d]	165.5 ± 76.5	182.2 ± 79.8	172.9 ± 50.7	0.164	0.793	0.536
RMSNz (A.U.)	442.7 ± 141.9[a]	300.6 ± 88.9[d]	265.5 ± 93.9[d]	338.6 ± 122.7	298.3 ± 117.7[d]	0.042	0.043	0.835
Burst (ms)	337.1 ± 23.9[b,d]	304.5 ± 29.8[c]	339.9 ± 36.0[d]	313.7 ± 36.0	342.6 ± 35.3[b,d]	0.305	0.005	0.754
TAINz (A.U.)	1089.7 ± 355.0	653.1 ± 274.8[d]	720.1 ± 323.5	739.6 ± 331.4	769.7 ± 238.9	0.082	0.510	0.575
MPF (Hz)	94.1 ± 9.8[b,c,d]	81.9 ± 14.1	79.6 ± 16.1	86.0 ± 19.7	89.5 ± 17.3[b,c]	0.007	0.769	0.221
MdPF (Hz)	80.5 ± 6.3[b,c,d]	66.3 ± 9.2	67.0 ± 13.5	71.3 ± 16.6	74.6 ± 14.0[b,c]	0.011	0.234	0.543

[a] *P < 0.05 compared with the other conditions.*
[b] *P < 0.05 compared with Nx10s.*
[c] *P < 0.05 compared with Nx60s.*
[d] *P < 0.05 compared with Hyp10s.*
RM ANOVA (2 × 2) Main Oxy, main oxygenation effect due to the conditions in which was performed the incremental exercise test (Nx, Normoxia; Hyp, hypoxia); RM ANOVA (2 × 2) Main Occ, Main Occlusion effect due to the duration of the occlusion (10 vs. 60 s); RMSraw, raw root mean square; RMSNz, Normalized root mean square; Burst, burst duration; TAINz, Normalized total activation index (arbitrary units, A.U.); MPF, mean power frequency; MdPF, median power frequency.

Muscle Metabolites

Muscle metabolites and the aerobic and anaerobic energy yield during the sprints have been reported elsewhere (Morales-Alamo et al., 2015). Briefly, ATP and PCr concentrations were reduced to a similar extent both in normoxia and hypoxia (ANOVA time effect: $P < 0.05$). From the 10th to 60th s of ischemic recovery ATP concentration remained unchanged while PCr declined an additional ∼5% (ANOVA time effect: $P < 0.05$ compared to Post and $P < 0.001$, compared to PRE). Muscle lactate concentration was increased to similar values 10 s after both incremental exercise tests (93.5 ± 24.3 and 88.3 ± 26.6 mmol kg d.w.$^{-1}$, in normoxia and hypoxia, respectively (ANOVA time effect: $P < 0.001$). From the 10th to 60th s of ischemia muscle lactate was increased by 24.0 ± 20.7 and 21.6 ± 24.5 mmol kg d.w.$^{-1}$, and muscle pH reduced by 0.102 ± 0.040 and 0.109 ± 0.041 units, after the IE in normoxia and hypoxia, respectively (ANOVA time effect: $P < 0.01$).

DISCUSSION

This investigation confirms, in agreement with previous studies using constant intensity (Marcora and Staiano, 2010) and incremental exercise to exhaustion in normoxia (Coelho et al., 2015), that task failure during incremental exercise in normoxia is not caused by muscle fatigue. The present study extends these findings to whole-body incremental exercise in severe hypoxia. We have also shown that muscle activation during the last 10 s of an incremental exercise to exhaustion is lower in severe hypoxia than in normoxia. This reduction in muscle activation cannot be explained by differences in metabolite accumulation between hypoxia and normoxia, and likely reflects central mechanisms of fatigue, which recovered, at least partly, during the next 10 or 60 s of ischemia, as reflected by the greater activation of the muscles during the subsequent sprint. The latter occurred despite the lower energy availability and the greater accumulation of metabolites after the ischemic recoveries. We have shown that

neuromuscular performance, as assessed by a sprint test, at the end of an incremental exercise to exhaustion, is greater when the incremental exercise has been performed in severe acute hypoxia than in normoxia despite similar muscle metabolite accumulation. This is also compatible with the incremental exercise in hypoxia ending by central mechanism/s acting earlier, i.e., with a lower amount of peripheral fatigue in hypoxia than in normoxia. This reduction in sprint performance is accompanied by a greater reduction in muscle activation (as reflected by the EMG_{RMS} changes) in the sprints carried out after IE in normoxia than hypoxia. This could also be interpreted as indicative of slower recovery of central mechanisms of fatigue during the 10 s of ischemic recovery that followed the IE performed in normoxia compared to the sprint performed after the IE in hypoxia.

Strikingly, following 60 s of recovery with complete occlusion of the circulation sprint performance was higher than that observed immediately following 10 s of occlusion, as previously reported (Morales-Alamo et al., 2015). This improvement in performance was achieved with lower EMG_{RMS} compared to the sprints executed after 10 s of ischemia. Thus, this investigation demonstrates dissociation between the recovery of the EMG_{RMS} and the recovery of power output. Since occlusion resulted in an increase of muscle lactate and H^+ accumulation from 10 to 60 s of occlusion (Morales-Alamo et al., 2015), the present experiments support the concept that muscle acidification contributes to reduce EMG_{RMS}. In addition, we have demonstrated that despite similar muscle concentrations of lactate and H^+ at the beginning of the respective sprints, the MPF and MdPF during sprint exercise are reduced by muscle fatigue when the sprint is performed after an incremental exercise in normoxia, but not in hypoxia. MPF and MdPF did not recover from the end of the incremental exercise to the start of the sprints, or from the 10th to the 60th second of ischemia, despite cessation of neural activation and appropriate oxygenation of the brain. These results point to peripheral mechanisms being primarily responsible for the reduction of MPF and MdPF during the sprints post-IE (Juel, 1988; Brody et al., 1991). Moreover, despite greater lactate and H^+ accumulation after 60 s of ischemic recovery, sprint exercise MPF and MdPF were not further reduced; therefore these two metabolites do not seem to account for the observed reduction of MPF and MdPF with muscle fatigue (Vestergaard-Poulsen et al., 1995). We have also shown that the duration of the bursts is reduced in fatigued muscles contracting under isokinetic conditions, recovering to non-fatigued levels within 60 s, regardless of the muscular concentrations of lactate and H^+. We lastly provided evidence for a minor impact, if any, of increased group III/IV afferent feedback on sprint exercise performance.

Surface EMG Changes are More Sensitive to Central than Peripheral Fatigue

To demonstrate the existence of central fatigue it is necessary to show that during a maximal voluntary contraction, direct stimulation of motor neural pathways or cortical motoneurons results in greater levels of force than elicited voluntarily (Merton, 1954; Gandevia et al., 1996). These procedures have several constraints limiting their application to complex tasks, such as pedaling. To circumvent these problems, a commonly used approach has been to carry out potentiated and interpolated twitch assessments at exhaustion as fast as possible, i.e., 1–2 min after the task failure, when most of the reduction in power output has already been recovered (Sargeant and Dolan, 1987; Fernandez-del-Olmo et al., 2013; Froyd et al., 2013; Coelho et al., 2015). Another limitation of stimulation techniques is that muscle fatigue is task-specific (Gandevia, 2001). Stimulation techniques cannot reproduce the complexity of motor orders involving thousands of motor units from different muscles firing at different rates, which are recruited with specific timings to achieve a coordinated movement. Also, in fatigued muscles, the increase in force elicited by an interpolated twitch may be due in part to intracellular mechanisms (i.e., a peripheral mechanism that is likely related to the force/$[Ca^{2+}]_i$ relationship) (Gandevia et al., 2013), not necessarily reflecting increased central fatigue.

An alternative approach for assessing muscle fatigue is to measure the maximal power that can be generated during the task that elicits muscle fatigue (Cairns et al., 2005; Marcora and Staiano, 2010; Coelho et al., 2015). However, during whole-body exercise on the cycle ergometer, pedaling frequency slows down close to task failure (Torres-Peralta et al., 2014). Given the dependency of power on muscle contraction velocity, fatigue should ideally be tested under similar muscle contraction velocities as during isokinetic pedaling (Sargeant and Dolan, 1987).

In the present experiments, the sprints were performed in isokinetic conditions, i.e., the duration of each pedaling cycle was always the same, regardless of the state of muscle fatigue (**Figure 2**). This isokinetic approach was possible because the cycle ergometer servo-control instantaneously varied the resistance applied to the pedals depending on the force exerted resulting in a constant pedaling rate of 80 rpm. These conditions allowed us to examine the impact of fatigue on certain components of the EMG signal without the variability induced by the speed of movement.

At a given pedaling rate, the duration of contraction bursts increases with the intensity of exercise, reaching maximal values at intensities close to Wmax (Torres-Peralta et al., 2014). In all conditions and every subject, the mean intensity achieved during the 10 s sprints was above the intensity reached at exhaustion during the IEs. This result implies that concerning intensity, burst duration should have been maximal during all sprints. However, burst duration during the sprints performed after 10 s of ischemic recovery was reduced, meaning that muscles were activated during a shorter fraction of the pedaling cycle compared to the control sprints. This effect might have been caused by reduced sarcolemmal excitability as a consequence of fatigue (Sejersted and Sjogaard, 2000; Sidhu et al., 2012). Reduced sarcolemmal excitability during hand grip exercise has been associated with lactic acidosis in blood (Hilbert et al., 2012). However, if present at exhaustion, sarcolemmal excitability appears to recover in less than 60 s after a fatiguing sprint exercise (Fernandez-del-Olmo et al., 2013). In agreement, burst duration recovered within the 60 s of ischemic rest after both IEs. Despite the observed recovery of burst duration, the EMG_{RMS} was further reduced following 60 s of ischemia. Since the reduction in EMG_{RMS}

was accompanied by increased power output and normal burst duration, it seems unlikely that the lower sprint EMG_{RMS} after 60 s of ischemia originates from a failure of the muscle cells to respond to neural activation.

During high-intensity contractions, the myosin ATPase and ion pumps account for most of the energy expenditure in muscles because no energy can be diverted to biosynthetic processes as the required enzymes are blocked (Morales-Alamo and Calbet, 2014). In our experimental conditions, upon cessation of incremental exercise the ion pumps are expected to be maximally activated, particularly the Na^+-K^+ pump, which has a critical role in restoring sarcolemmal excitability (Pedersen et al., 2003; Hostrup et al., 2014). During ischemia, the energy needed to maintain the activity of the ion pumps was provided by the glycolysis and to a lesser extent by the minute amount of remaining PCr (Morales-Alamo et al., 2015). The O_2 that still remained bound to myoglobin at the end of incremental exercise was rapidly used upon occlusion due to the strong activation of mitochondrial respiration by the increased ADP concentration at task failure (Morales-Alamo et al., 2015). Femoral vein and mean capillary PO_2 were lower at exhaustion after the incremental exercise test in severe hypoxia than in normoxia (Calbet et al., 2015). Therefore, the potential contribution of the small amount of O_2 trapped in the occluded leg (or remaining bound to myoglobin) to ATP re-synthesis should have been lower in hypoxia than in normoxia. However, although more O_2 was available at exhaustion for the initial recovery in normoxia, i.e., within the first 10 s of occlusion, performance was more impaired in the sprint performed after 10 s of ischemic recovery that followed the normoxic rather than the hypoxic IEs. This result concurs with task failure occurring with a lower level of peripheral fatigue during IE in hypoxia than normoxia, as our results indicate.

Studies in cats (Hill et al., 1992; Lagier-Tessonnier et al., 1993) and rabbits (Arbogast et al., 2000) have shown increased baseline discharge frequency of group III and IV muscle afferents by PO_2 close to the values observed in the femoral vein in the present investigation (Calbet et al., 2015). Thus, lower interstitial PO_2 values in hypoxia than normoxia could have enhanced III/IV afferent feedback to cause increased inhibition of the corticospinal drive during exercise in severe acute hypoxia, as previously suggested (Calbet et al., 2015). Muscle III and IV afferent, and perhaps other sensory endings in joints and tendons, have been postulated to contribute to central fatigue (Reid, 1927; Bigland-Ritchie et al., 1986; Garland, 1991; Amann et al., 2008, 2013a). Animal studies have shown that when motoneurons are stimulated repetitively many neurons reduce their discharge frequency or stop firing (Kernell and Monster, 1982a,b; Spielmann et al., 1993). This phenomenon is likely accentuated by hypoxia. Consequently, reduced responsiveness of some motoneurons pools (central fatigue) combined with increased III/IV muscle afferent feedback and increased ventilatory demand at any given absolute intensity might have increased the perception of effort (Marcora, 2009), leading to task failure at the end of IE in hypoxia with a lower level of peripheral fatigue than in normoxia (Pierrefiche et al., 1997). Oxygenation upon exhaustion might have restored faster central fatigue at the end of the IE in hypoxia than normoxia, by reinstating within seconds normoxic interstitial PO_2 levels in the CNS. In agreement, with altitude acclimatization, oxygen delivery at VO_2max is almost restored to sea level values (Calbet and Lundby, 2009), improving brain oxygenation compared to acute hypoxia and reducing supraspinal fatigue (Goodall et al., 2014a,b).

The absolute exercise intensity at task failure was lower in hypoxia than normoxia. However, metabolite accumulation was similar regardless of F_IO_2 and, hence, differences in absolute exercise intensity do not seem to account for the differences in peripheral fatigue.

Group III and IV Muscle Afferent Stimulation does not Limit Peak Power Output during Whole-Body Sprint Exercise in Healthy Humans

Group III/IV muscle afferent neurons include a complex family of afferent endings some of which act as nociceptors while others respond to thermal, mechanical or chemical stimulation (Light et al., 2008; McCord and Kaufman, 2010; Jankowski et al., 2013). Metabolic products of muscle contraction like H^+ (Rotto and Kaufman, 1988; Light et al., 2008), lactate (Light et al., 2008), adenosine (Middlekauff et al., 1997), ATP (Light et al., 2008), nitric oxide (Arbogast et al., 2001), and inflammation mediators may also increase III/IV muscle afferent discharge (Light et al., 2008; McCord and Kaufman, 2010). It is particularly important that the response to the isolated increase of H^+, ATP and lactate is much lower than observed to the combination of the three (Light et al., 2008). Some metaboreceptive III and IV muscle afferents seem specialized in detecting innocuous levels of metabolites, while others respond to noxious levels and contribute to muscle pain (Kniffki et al., 1978; Mense, 1996). It is thought that both low and high concentration responding-endings could contribute to sympathetic reflexes and to increase the perception of effort (Light et al., 2008).

Using lumbar intrathecal fentanyl administration before exercise to block μ-opioid receptor-sensitive group, Amann and co-workers (Amann et al., 2009, 2011) studied the influence of group III and IV afferents on voluntary activation (assessed by the twitch-interpolation technique) after constant-intensity bicycling exercise. After 3 min of recovery, voluntary activation was reduced by 1.2% during the placebo trial (non-significant) and 1.7% during the fentanyl trial (Amann et al., 2011). Moreover, 3 min after a time trial that caused fatigue in 7.5 min, voluntary activation was reduced by 1.6% (compared to 0.8% in the placebo trial) (Amann et al., 2009). These results further emphasize that any potentially negative influence of III and IV muscle afferents on voluntary activation is likely small after whole-body exercise or that voluntary activation capacity recovers rather quickly (Bigland-Ritchie et al., 1986; Kennedy et al., 2015; Pageaux et al., 2015).

Amann et al. (2011) reported that despite the inhibition of III and IV muscle afferents, fentanyl markedly reduced performance (from 8.7 to 6.8 min) during a constant-intensity trial to exhaustion. In another study by the same group

(Amann et al., 2009), where the subjects performed a time trial, performance was similar in the fentanyl and placebo experiments. The reason for these discrepant effects of III/IV muscle afferents inhibition on performance is not clear (Marcora, 2010). In the case of the constant-intensity trial, there was a substantial impairment in O_2 transport in the fentanyl trial, an effect caused by reduced ventilation, leading to lower hemoglobin saturation and VO_2 with fentanyl than placebo. A slightly lower impairment of O_2 transport was reported in the time trial experiments, which could have been compensated for by increased involvement of the anaerobic metabolism (Amann et al., 2009). Marcora (2010) have suggested that during the time trial, the "belief effect" induced by reducing leg muscle pain with fentanyl-induced a fast start, which compensated for the negative effect of fentanyl on cardiorespiratory responses. As a result, exercise performance was unchanged during the time trial. During the constant-intensity trial, the "belief effect" could not induce a fast start and consequently exercise performance was reduced.

Interestingly, in both experiments with fentanyl the reduction of quadriceps potentiated twitch forces elicited by magnetic neural stimulation were greater than in the control trials (Amann et al., 2009, 2011). Assuming that a reduction in the force elicited by potentiated twitches indicates a greater level of peripheral fatigue and that the subjects exercised to their limits in all trials, Amann and co-workers studies give support to the concept that III/IV afferent feedback is used to set the limit of peripheral fatigue that is permitted. However, these experiments, combined with the present findings, show that task failure at the end of an IE to exhaustion (current study), or at the end of constant-intensity (Marcora and Staiano, 2010; Bosio et al., 2012) or a simulated time-trial competition (Amann and co-workers studies) is not due to a peripheral failure. Both types of experiments show that greater levels of peripheral fatigue are possible, but not reached because the exercise ends due to central mechanisms of fatigue (Kayser, 2003). The critical question is whether peripheral fatigue causes central fatigue and task failure through a central mechanism by activating the metaboreflex. If the latter is the main mechanism causing central fatigue and task failure a reduction in sprint performance under conditions with increased metaboreflex activation will be expected. However, our results show that this is not the case.

Metabolite accumulation at task failure and during ischemia should have promoted a sustained discharge of III/IV muscle afferents (Darques and Jammes, 1997; Darques et al., 1998; Light et al., 2008; McCord and Kaufman, 2010), an effect that would be expected to be greater after 60 than 10 s occlusions in the present investigation. Nevertheless, sprint exercise power output was higher after 60 than 10 s of ischemia. Increased metaboreflex activation should exacerbate the ventilatory response to exercise (Amann et al., 2010), as observed during the sprints performed following 60 s of ischemia in the present investigation (Morales-Alamo et al., 2015). Thus, despite increased metaboreflex activation during the sprints after 60 s of ischemia, and a theoretically worsened metabolic situation, peak and mean power output was higher during the sprints after 60 than 10 s of ischemia. Our findings appear to be at odds with the recent study by Kennedy et al. (2015), in which the occlusion of the circulation during 2 min after a 2-min sustained MVC of the knee extensors resulted in a progressive reduction of the maximal voluntary contraction (MVC) (see Figure 3A of Kennedy et al., 2015). This effect was accompanied by a progressive reduction of maximal voluntary activation indicative of increasing central fatigue (see Figure 3B of Kennedy et al., 2015). In agreement with our results, Kennedy et al. (2015) observed a fast recovery of voluntary activation upon release of the cuff, indicating that the negative influence of III/IV afferent discharge on voluntary activation was also relieved very rapidly. This quick response could be due to (i) the release of the direct compression on the femoral nerve, (ii) the washout of metabolites combined with oxygenation due to reactive hyperemia, or (iii) the combination of these effects. In our experimental conditions, the hyperemic response to the release of the cuff was likely much greater than in Kennedy et al. (2015) as reflected by the rapid increase of pulmonary VO_2 and the elevated heart rate at the beginning of the sprint (Morales-Alamo et al., 2015). Another interesting aspect of the study by Kennedy et al. (2015) is that the level of peripheral fatigue as assessed by potentiated twitches remained at the same level during the 2 min of ischemia, despite 5 × 2-s long MVC maneuvers (i.e., 10 s of maximal contractile activity with occlusion of the circulation). Thus, the 2 min of post-exercise ischemia did not worsen peripheral fatigue (Kennedy et al., 2015). Although Kennedy et al. (2015) did not measure muscle metabolites, 10 s of maximal contractile activity during the 2 min of ischemia, in combination with increased metabolic demand due to the preceding MVC (sustained during 2 min), should have increased peripheral fatigue according to the prevailing paradigm.

The present investigation strongly suggests that the inhibitory role of muscle afferent discharge on the corticospinal motor drive during maximal intensity whole-body exercise is either small or counteracted by a strong corticospinal drive (central command). Otherwise, the III/IV muscle afferent feedback due to ischemia (PO_2 close to 0 mmHg in our experimental conditions) and the metabolites accumulated during the IEs should have decreased Wpeak and Wmean during the 10 or 60 s post-ischemia sprints below the Wmax achieved at task failure in the IEs. This finding contrasts with experiments using single joint (Gandevia et al., 1996; Kennedy et al., 2015) or handgrip dynamic contractions (Broxterman et al., 2015), where a clear inhibition of voluntary activation is consistently shown.

In the present investigation, increased III/IV muscle afferent feedback could explain why the observed EMG_{RMS} were lower in the sprints performed after 60 s than after 10 s of ischemia. Likewise, that MPF and MdPF remained at task failure levels during the post-IE sprints is also consistent with an on-going negative feedback reducing motoneurons firing frequencies (Broxterman et al., 2015). However, the power output achieved during the sprints after 60 s of ischemia is close to the maximal power attainable by our subjects without phosphagens (Morales-Alamo et al., 2015), leaving little room for central mechanisms

to limit sprint performance after 60 s of ischemia. Thus, it seems that 60 s of ischemic recovery allows for restoration of the central mechanisms of fatigue, despite the discharge from the III/IV muscle afferents. The latter was likely counteracted by a strong central command and a rapid reperfusion of the muscle upon the release of the cuff during the post-IE sprints. In agreement with this interpretation, Amann et al. (2009) reported no effect of intrathecal fentanyl on time trial performance, although the power output profile was dramatically changed.

Reduced Sprint EMG_{RMS} But Increased Power Output after 60 than 10 s Post-Exercise Ischemia

In vitro experiments have shown that lactate and H^+ accumulation may facilitate peripheral recovery by increasing chloride conductance (Nielsen et al., 2001; Pedersen et al., 2003; Karelis et al., 2004). Thus, an elevated glycolytic rate combined with a progressive rise of muscle temperature due to flow arrest during ischemic recovery could have exerted two opposing actions. Positively, increased glycolysis may have facilitated peripheral recovery by enhancing sarcolemmal excitability (Pedersen et al., 2003), which was likely depressed immediately after the IEs. Negatively, increased glycolysis and the subsequent metabolite accumulation may have increased III/IV muscle afferent discharge, interfering with muscle activation. The combination of both mechanisms could explain the lower EMG_{RMS} values observed during the sprints performed 60 s compared to 10 s after the IEs. If we assume that the normalized EMG_{RMS} is a valid index of corticospinal motor output, only reduced when the corticospinal motor drive is lower, a greater reduction in sprint EMG_{RMS} after prolonged (60 s) compared to shorter (10 s) ischemia would lead to the untenable conclusion that all of the improvement in Wpeak and Wmean observed from 10 to 60 s of ischemic recovery is due to peripheral mechanisms. Moreover, this peripheral-based improvement would have to be sufficient to counteract the reduced motor command. A more plausible explanation is that for a given level of neural activation, EMG_{RMS} is reduced by metabolite accumulation; i.e., EMG_{RMS} does not faithfully reflect the level of neural activation (Vestergaard-Poulsen et al., 1995). In agreement with this interpretation, it has been shown that central fatigue recovers rapidly upon cessation of contractile activity, even when peripheral fatigue remains at the same level (Bigland-Ritchie et al., 1986; Gandevia et al., 1996). Therefore, the observed reduction in sprint EMG_{RMS} after 60 s compared to 10 s of ischemic recovery is unlikely to be caused only by increased central mechanisms of fatigue.

Fatigue Reduces the Duration of the Burst during Isokinetic Sprints

The duration of the burst was similarly decreased in the sprints performed 10 s after the incremental exercise in hypoxia and normoxia. However, sprint power output was greater after the incremental exercise in hypoxia. To generate greater power, more mechanical impulse must be produced. As the duration of the bursts (a surrogate of contraction time) was similar in the sprints performed 10 s after both IEs, the subjects must have applied a greater force on the pedals after the hypoxic IE. Therefore, greater motor cortical drive was required in the sprint that occurred after hypoxia. In agreement, with this conclusion, the MPF and MdPF were higher in the sprints preceded by IE in hypoxia than normoxia, suggesting greater firing rates (Solomonow et al., 1990; Gerdle and Fugl-Meyer, 1992; Sbriccoli et al., 2003) and a lower degree of central fatigue during the sprint 10 s after the IE in hypoxia. Alternatively, central fatigue could have recovered more rapidly after the IE in hypoxia, likely due to a direct effect of oxygenation on the CNS, as previously postulated (Kayser et al., 1994; Calbet et al., 2003a,b; Amann et al., 2007; Goodall et al., 2014a).

Limitations

At the end of the incremental exercise muscle activation was much lower than during the subsequent sprints, despite the fact muscles remained ischemic and that metabolite accumulation reduces EMG_{RMS}. This finding indicates that task failure was not due to muscle fatigue; it was caused by reduced muscle activation. However, this conclusion is not based on genuine assessments of central fatigue and peripheral fatigue with stimulation techniques. Nonetheless, there is not an ideal method to quantify the extent of change in neuromuscular function (Cairns et al., 2005). Current methods based on stimulation techniques have also limitations. For example, the response of fatigued muscles to potentiated twitches depends on the method of stimulation (Froyd et al., 2013). Moreover, methods based on electrical or magnetic stimulation may give results different from those obtained using voluntary dynamic contractions. Few minutes after a 30 s all out sprint exercise (Wingate test) potentiated quadriceps twitches indicate substantial peripheral fatigue, when at the same time peak power output has completely recovered (Fernandez-del-Olmo et al., 2013).

In summary, task failure at the end of an incremental exercise to exhaustion depends more on central than peripheral mechanisms, as indicated by the fact that the level of muscle activation observed during the last 10 s of the incremental exercise was much lower than that reached 10 s later during a 10 s sprint, despite the fact that muscle remained ischemic from the end of exercise to the start of the sprint. The central components of fatigue appear to recover to a greater extent, during the first 10 s following an incremental exercise to exhaustion in hypoxia than in normoxia, concomitant with a rapid change to normoxic breathing during the recovery. We have also shown that ischemic recovery after incremental exercise to exhaustion allows for a partial restoration of power output despite increased acidification and reduced EMG_{RMS}. Moreover, this study demonstrates that MPF, MdPF and the duration of the bursts during isokinetic sprints are reduced with fatigue. Lastly, this investigation indicates that increased group III/IV muscle afferent discharge has a minor, if any, negative impact on sprint exercise performance in healthy humans.

AUTHOR CONTRIBUTIONS

Conception and design of the experiments: JC; pre-testing, experimental preparation, data collection and analysis: RT, DM, JL, IP, and JC; EMG analysis: RT, MG, and MI. The first version of the manuscript was written by RT and JC. All co-authors read, contributed comments and approved the final version of the manuscript.

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Increased P_IO_2 at Exhaustion in Hypoxia Enhances Muscle Activation and Swiftly Relieves Fatigue: A Placebo or a P_IO_2 Dependent Effect?

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To determine the level of hypoxia from which muscle activation (MA) is reduced during incremental exercise to exhaustion (IE), and the role played by P_IO_2 in this process, ten volunteers (21 ± 2 years) performed four IE in severe acute hypoxia (SAH) (P_IO_2 = 73 mmHg). Upon exhaustion, subjects were asked to continue exercising while the breathing gas mixture was swiftly changed to a placebo (73 mmHg) or to a higher P_IO_2 (82, 92, 99, and 142 mmHg), and the IE continued until a new exhaustion. At the second exhaustion, the breathing gas was changed to room air (normoxia) and the IE continued until the final exhaustion. MA, as reflected by the *vastus medialis* (VM) and *lateralis* (VL) EMG raw and normalized root mean square (RMSraw, and RMSNz, respectively), normalized total activation index (TAINz), and burst duration were 8–20% lower at exhaustion in SAH than in normoxia ($P < 0.05$). The switch to a placebo or higher P_IO_2 allowed for the continuation of exercise in all instances. RMSraw, RMSNz, and TAINz were increased by 5–11% when the P_IO_2 was raised from 73 to 92, or 99 mmHg, and VL and VM averaged RMSraw by 7% when the P_IO_2 was elevated from 73 to 142 mmHg ($P < 0.05$). The increase of VM-VL average RMSraw was linearly related to the increase in P_IO_2, during the transition from SAH to higher P_IO_2 ($R^2 = 0.915$, $P < 0.05$). In conclusion, increased P_IO_2 at exhaustion reduces fatigue and allows for the continuation of exercise in moderate and SAH, regardless of the effects of P_IO_2 on MA. At task failure, MA is increased during the first 10 s of increased P_IO_2 when the IE is performed at a P_IO_2 close to 73 mmHg and the P_IO_2 is increased to 92 mmHg or higher. Overall, these findings indicate that one of the central mechanisms by which severe hypoxia may cause central fatigue and task failure is by reducing the capacity for reaching the appropriate level of MA to sustain the task. The fact that at exhaustion in severe hypoxia the exercise was continued with the placebo-gas mixture demonstrates that this central mechanism has a cognitive component.

Keywords: fatigue, performance, hypoxia, altitude, muscle activation, human experimentation, exercise, oxygenation

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INTRODUCTION

Muscle activation, as reflected by the root mean square of the electromyographic signal (EMG_{RMS}), is higher in severe acute hypoxia (SAH) than normoxia at the same absolute intensity, but lower in hypoxia than in normoxia at the same relative intensity (Torres-Peralta et al., 2014). Close to exhaustion, the surface integrated electromyographic (iEMG) activity is higher during constant-intensity exercise in hyperoxia ($F_IO_2 = 0.30$) than in SAH ($F_IO_2 = 0.10$) (Amann et al., 2007). This could mean that hypoxia limits the motor drive output from the central nervous system (CNS) leading to reduced muscle activation (MA) and task failure. In agreement with this idea, during exercise in severe hypoxia, fatigue is rapidly relieved by oxygenation with normoxic (Calbet et al., 2003a) or hyperoxic gas (Amann et al., 2007). If hypoxia depresses muscle activation, oxygenation should be accompanied by an immediate increase in MA while the intensity of exercise remains at the same absolute level. However, it remains unknown whether the ergogenic effect of an increase in oxygenation requires a concomitant elevation of muscle activation.

During exercise in severe acute (Calbet et al., 2003a, 2015a; Amann et al., 2007; Morales-Alamo et al., 2015) and chronic hypoxia (Kayser et al., 1994; Calbet et al., 2003b) task failure is thought to be predominantly caused by central mechanisms sensitive to reduced O_2 delivery to the brain (Goodall et al., 2012, 2014) and to reduced interstitial brain PO_2 (Amann and Calbet, 2008). A fundamental difference between exercise in severe and moderate hypoxia is the region of the hemoglobin oxygen dissociation curve (ODC) at which the gas exchange occurs in the lungs. In severe hypoxia, pulmonary gas exchange occurs in the straight region of the ODC, implying that a small increase in arterial oxygen pressure (PaO_2) would result in a greater elevation of arterial hemoglobin saturation (SaO_2) (Calbet et al., 2003a; Calbet and Lundby, 2009). In moderate hypoxia, pulmonary gas exchange occurs at the upper and flatter region of the ODC, where an improvement in PaO_2 translates into a smaller elevation of SaO_2 (Amann et al., 2007). The fact that increasing inspiratory oxygen pressure (P_IO_2) to hyperoxic levels only relieved fatigue when applied at exhaustion in severe hypoxia could indicate that a substantial elevation of arterial oxygen content (CaO_2) is required (Amann et al., 2007). However, the observation by Amann et al. (2007) that increased P_IO_2 does not relieve fatigue during moderate hypoxia could indicate that the increase in SaO_2 is even more critical than the elevation of PaO_2, since in the flatter region of the ODC the improvement of SaO_2 for a given increase of PaO_2 is smaller. It remains unknown what levels of improvement in PaO_2 and CaO_2 are required to relieve fatigue and enhance the neural activation of muscles upon exhaustion in hypoxia.

Therefore, the aims of this study were to (a) determine the influence of the level of hypoxia on a potential reduction of MA at exhaustion; (b) determine the minimum increase in P_IO_2 needed to enhance muscle activation at exhaustion in hypoxia; and (c) find out if the ergogenic effect of increasing P_IO_2 is always accompanied by enhanced muscle activation, which would be an indication of a predominantly central mechanism.

We hypothesized that an increase of P_IO_2 upon exhaustion would rapidly increase MA depending on the level of hypoxia at exhaustion and the inspiratory O_2 pressure of the breathing gas.

MATERIALS AND METHODS

Subjects

Ten healthy men (age: 21.1 ± 2.1 years, height: 173 ± 8 cm, body mass: 71 ± 9 kg, body fat: 16.6 ± 4.5%, VO_2max: 50.4 ± 4.7 $mL.kg^{-1}.min^{-1}$) agreed to participate in this investigation. After being informed about the experiments and the possible risks associated with participation they provided written consent. The study was performed by the Helsinki Declaration and was approved by the Ethical Committee of the University of Las Palmas de Gran Canaria (CEIH-2010-01 and CEIH-2009-01).

General Overview

This study was a part of a larger project that included several experiments designed to address the mechanisms limiting whole body exercise performance in humans. The results focusing on muscle metabolism and O_2 transport have been published (Calbet et al., 2015a; Morales-Alamo et al., 2015). Body composition was determined by dual-energy x-ray absorptiometry (DEXA) (Hologic QDR-1500, Hologic Corp., software version 7.10, Waltham, MA), during the familiarization sessions. The leg muscle mass was calculated from the DEXA scans using the model of Wang et al. (1999). Subjects reported to the laboratory to familiarize with maximal exercise tests in normoxia and normobaric hypoxia ($Altitrainer_{200}$, SMTEC, Switzerland) on separate days. For experimental purposes, subjects performed two sets of IE tests, here called *invasive* and *deception* test. On the first experimental day, all subjects performed the invasive tests as previously described (Calbet et al., 2015a) and on the second and third day, they completed the deception protocol. The exercise tests were carried out on a cycle ergometer (Lode Excalibur Sport 925900, Groningen, The Netherlands) and subjects were instructed to pedal at 80 revolutions per minute (rpm). To facilitate the maintenance of the targeted pedaling cadence, subjects received visual feedback, and verbal instructions when deviations of 5 or more rpm occurred.

Exercise Protocol

Invasive Experiments

Subjects reported to the laboratory at 07.00 after an overnight fast from 22.00 h. After catheterization (see below), subjects were assigned to either an IE test to exhaustion in normoxia (30 W/2 min) or hypoxia ($P_IO_2 = 73$ mmHg; 20 W/2 min; Altitrainer200, SMTEC, Switzerland), in random order and separated by 90 min rest. Before the start of the IE in hypoxia, subjects were breathing the hypoxic gas for 3 min while they were pedaling 20–40 rpm with the ergometer unloaded. At exhaustion (Exh1), the subjects were rapidly switched to breath room air (normoxia) and requested to continue the exercise at the same load for 2 min, then the load was increased by 20 W every 2 min until exhaustion (Exh2). This was followed by a lunch break (a sandwich and 200 mL of apple or pineapple juice) and a 120 min resting

period. Thereafter, the IE in hypoxia was repeated. At Exh1 the subjects were requested to keep pedaling while a valve deviated the inspired flow to a 30 L anesthesia bag pre-filled with hypoxic gas (F_IO_2 = ~13.3, P_IO_2 = ~91 mmHg) and a small amount of CO (7 mL·kg^{-1} body mass). The gas was breathed in an open circuit system in a well-ventilated room until the bag was almost emptied. The valve was then returned to the previous position such that the subjects continued the incremental test at this level of hypoxia (F_IO_2: ~13.3, P_IO_2: ~91 mmHg). After 2 min at the load eliciting exhaustion, the intensity was increased by 20 W/2 min until a new exhaustion (Exh2). Again, subjects were requested to keep pedaling while they were switched to breath room air (normoxia). After 2 min, the load was increased by 20 W/2 min until exhaustion (Exh3). The invasive experiments were used to study the influence of different levels of oxygenation on the hemodynamic responses and fatigue mechanisms in hypoxia, as reported previously (Calbet et al., 2015a).

Deception Protocol (Noninvasive)

Subjects performed four IE tests on 2 different days, separated by at least 1 week. A 90 min recovery period was established between the two tests carried out on the same day (**Figure 1**), as previously done (Calbet et al., 2003a). This resting period is sufficient to allow for a full recovery of peak power output and VO_2max, as previously reported (Scharhag-Rosenberger et al., 2014; Calbet et al., 2015a). Each deception test was composed of an initial phase in severe hypoxia (P_IO_2 = 73 mmHg) (HYP1), followed by a second phase with a similar or a less severe level of hypoxia (HYP2), which continued with a final phase in normoxia (NX3). HYP1 started with an intensity of 60 or 70 W which, after 2 min was increased by 20 or 30 W every 2 min until exhaustion (Exh1). The 70 W starting load and the steps of 30 W were used in one of the subjects who was a well-trained triathlete, so the duration of his test was similar to the duration of the tests performed by the other subjects. Like during the invasive experiments, before the start of the IE in hypoxia, subjects were breathing the hypoxic gas for 3 min while they were pedaling at 20–40 rpm with the ergometer unloaded. At Exh1, the inspired gas mixture was rapidly changed to one of four different gas mixtures [P_IO_2 = 73 (placebo), 82, 92, and 99 mmHg, equivalent to 5200, 4400, 3600, and 3100 m above sea level, respectively]. Subjects were told and believed that they were getting normoxic gas at exhaustion. These gas mixtures were administered in random order and with a double-blind design. After 2 min at the load eliciting Exh1, the load was increased by 20 or 30 W every 2 min until exhaustion (Exh2). At Exh2, the gas mixture was rapidly changed to room air (P_IO_2 = 142 mmHg) while the subjects were strongly encouraged to continue pedaling. After 2 min at the load eliciting Exh2, the load was increased by 20 or 30 W every 2 min until exhaustion (Exh3). Although the change of P_IO_2 upon exhaustion was intended to be maintained for 2 min before increasing the load, in some instances, for example during the placebo experiments, subjects fatigued before reaching 2 min in the new oxygenation condition. In these cases, the breathing gas mixture was rapidly changed to normoxia, maintained for 2 min in normoxia, and then increased by 20 or 30 W every 2 min until exhaustion. Exhaustion during the IE tests was defined by either the subject stopping pedaling or dropping pedaling rate below 60 rpm during 5 s (or earlier if the cadence was dropping very fast), despite strong verbal encouragement. A 30 L anesthesia bag was prefilled with the target F_IO_2 and used as a buffer in the transition to HYP2, to gain few seconds to adjust the Altitrainer in such a way that the target F_IO_2 was instantaneously administered at the start of the transition. During the first 10–12 s of the transitions the subjects breathed from the anesthesia bag, then a four-way valve was used to direct the inspiratory port to either the Altitrainer or room air. These 10 s (bag breathing) were used to stabilize the Altitrainer at the target F_IO_2 corresponding to each HYP2 phase.

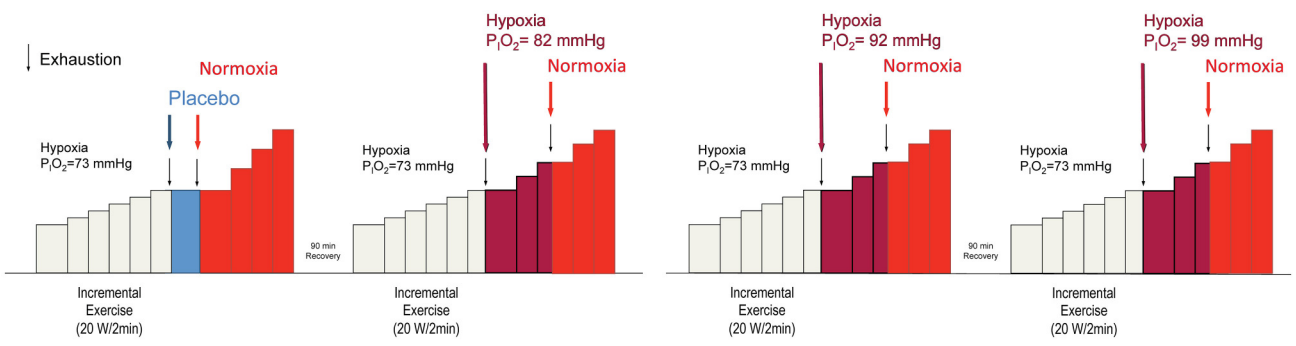

FIGURE 1 | Experimental protocol. Each experimental day the subjects performed two incremental exercise tests in random order. The incremental exercise test always began in severe hypoxia (P_IO_2 = 73 mmHg). At exhaustion (Exh1) the breathing gas mixture was swiftly changed to another one with a greater oxygen PO_2, except in one instance that the gas administered was the same one the subjects were breathing in severe acute hypoxia, i.e., P_IO_2 = 73 mmHg, to create a placebo condition. Subjects were asked to continue the exercise and after 2 min at the load eliciting Exh1, the load was increased by 20 or 30 W/2 min until exhaustion (Exh2). Once again, subjects were asked to keep pedaling while the gas mixture was swiftly changed to normoxia. After 2 min at the load eliciting Exh2, the intensity was increased by 20 or 30 W/2 min until the final exhaustion (Exh 3). Between Exh1 and Exh2, the breathing gas mixtures used corresponded to P_IO_2 of 73, 82, 92, 99, and 142 mmHg (Normoxia) and were administered following a double-blind design. Subjects were asked to pedal close to 80 rpm. However, when approaching exhaustion, the pedaling rate was always reduced.

Oxygen Uptake and Hemoglobin Oxygen Saturation

Oxygen uptake was measured with a metabolic cart (Vmax N29; Sensormedics, California, USA), calibrated before each test according to the manufacturer instructions. Respiratory variables were analyzed breath-by-breath and averaged every 10 s for the analysis of transitions at exhaustion. Hemoglobin oxygen saturation was estimated with a finger pulse oximeter (SpO_2) (OEM III module, 4549-000, Plymouth, MN).

Electromyography

Electrical MA was monitored using surface electromyography (EMG) (**Figure 2**). EMG signals were continuously recorded from the *vastus medialis* and *vastus lateralis*, as previously reported (Torres-Peralta et al., 2016). Before the application of the EMG electrodes, the skin surface was carefully shaved, and wiped with ethanol to reduce skin impedance. Bipolar single differential electrodes were placed longitudinally on the muscles following the SENIAM recommendations (Merletti and Hermens, 2000) and taped on the skin to minimize movement artifacts. The reference electrode was placed on the skin over the acromion. The position of the electrodes was marked on the skin with indelible ink, and these references were used for precise electrode placement in repeated experiments.

The EMG signals were acquired using a 16-channel recording system (Myomonitor IV, Delsys Inc., Boston, MA) at a sampling rate of 1000 Hz using rectangular shaped (19.8 mm wide and 35 mm long) bipolar surface electrodes with 1 × 10 mm 99.9% Ag conductors, and with an inter-conductor distance of 10 mm (DE-2.3 Delsys Inc.). The EMG data were filtered with a high-pass filter of 20 Hz and a low-pass filter of 450 Hz using a fifth-order Butterworth filter. The system has an input impedance of $>10^{15}\Omega$ per 0.2pF of input capacitance, a common mode rejection ratio of >80 dB, signal-to-noise ratio < 1.2 μV, and a pre-amplifier gain 1000 V/V ±1 %. Each pedal revolution was detected using an electrogoniometer (Goniometer Biosignal Sensor S700 Joint Angle Shape Sensor; Delsys Inc. Boston) fixed on the left knee and sampled at 500 Hz. The electrogoniometer was individually calibrated taking as references the knee angles in fully extended and flexed positions. EMG and joint movement were simultaneously recorded by a portable device (Myomonitor IV, Delsys Inc. Boston) and wirelessly transmitted to a computer

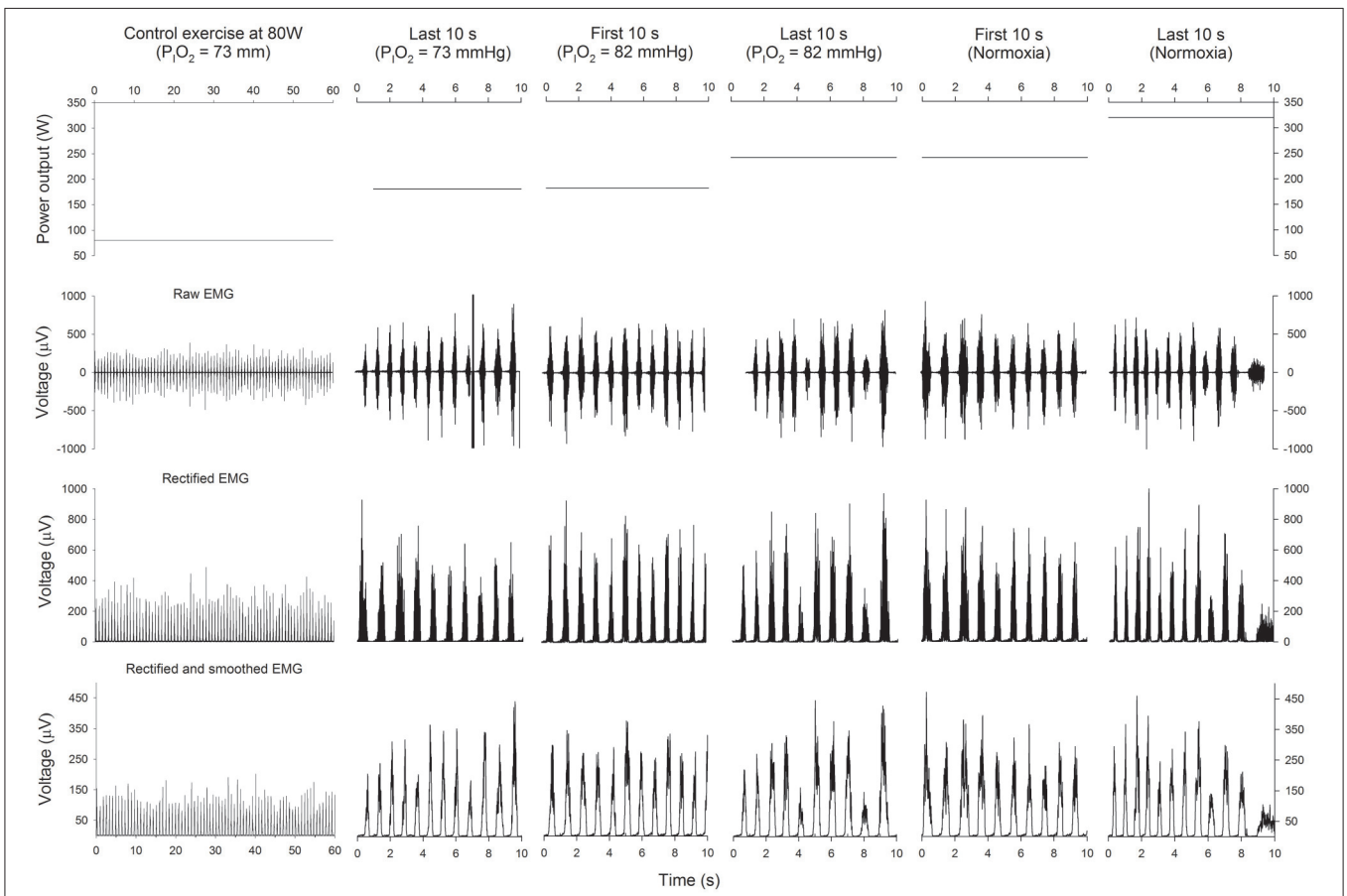

FIGURE 2 | Power output and EMG. Schematic representation of the power output (upper panels), raw EMG (2nd row), rectified EMG (3th row), and rectified and smoothed EMG (lower panels), during the last 60 s of the control submaximal exercise at 80 W in hypoxia (P_IO_2 = 73 mmHg), the last 10 s of the incremental exercise (IE) in severe hypoxia (P_IO_2 = 73 mmHg), the first 10 s of the transition from a P_IO_2 of 73 to 82 mmHg, the last 10 s of the IE at a P_IO_2 of 82 mmHg, the first 10 s in normoxia and the last 10 s before task failure in normoxia.

(EMGWorks Wireless application and EMGWorks Acquisition 3.7.1.3; Delsys, Inc. Boston).

The EMG signal corresponding to each muscle contraction was analyzed using code developed "in house" (Matlab R2012b, MathWorks, Natick, MA, USA). The EMG recordings were full-wave rectified and smoothed to provide an index of muscle activation; the amplitude characteristics were analyzed via average RMS of a 25-ms moving window for the duration of the contraction burst. Contraction burst onset and offset detection were determined using 20% of the maximal EMG_{RMS} activity of each contraction burst as a reference (Baum and Li, 2003; Hug and Dorel, 2009; Torres-Peralta et al., 2014), rather than a mean threshold value from 15 consecutive contraction bursts (Ozgunen et al., 2010). This approach yielded the same result as direct, simple visual discrimination, with 100% detection of all contraction bursts. Contraction timing was defined as the time elapsed from the knee at is greatest extension to the start of the contraction burst, expressed as a percentage of the full duration of each revolution. The EMG_{RMS} recorded during the last minute of a 2 min 80 W load (in hypoxia, P_IO_2 = 73 mmHg) was used to normalize the remaining EMG_{RMS} data. Besides, we defined a total activity index (TAI) as TAI = EMG_{RMS} × burst duration (ms) × number of pedal strokes during the period of time analyzed. The total activity index is similar to the integrated EMG signal, but was computed separately for each contraction burst and excluded the baseline EMG between contraction bursts (Torres-Peralta et al., 2014). The TAI recorded during the last minute of a 2 min 80 W load (in hypoxia) was used to normalize the rest of the TAI values.

The mean (MPF) and median (MdPF) power spectrum frequencies were calculated using Fast Fourier Transform (Solomonow et al., 1990). All variables were reported as the mean values of the pedal strokes recorded during the last 10 and 30 s of the incremental exercise. EMG data are reported separately for *vastus medialis* (VM) and *lateralis* (VL), and also as the average of the two muscles.

Calculation of the Improvement in SaO_2 during the First 10 s of the Transitions

The mean change in SaO_2 needed to explain the mean improvement in VO_2 observed during the first 10 s of the transition from hypoxia to higher a P_IO_2 was calculated by solving the Fick equation, using arterial blood gasses and thermodilution cardiac output data obtained in normoxia and hypoxia (P_IO_2 = 73 mmHg) in parallel invasive experiments performed by the same subjects (Calbet et al., 2015a). Since similar levels of peak cardiac output were reached in severe hypoxia and normoxia, it was assumed that the level of cardiac output reached at exhaustion at intermediate P_IO_2 levels (i.e., 82, 92, and 99 mmHg) must have been similar to that measured in normoxia. It was also assumed that cardiac output remained unchanged during the first 10 s of the transition, given the stability of heart rate during the transitions and the high dependency of cardiac output on the absolute exercise intensity (Calbet and Lundby, 2009; Calbet et al., 2009a, 2015b), which remained unchanged during the first 10 s of the transition.

Statistics

Normal distribution of variables was checked using the Shapiro-Wilks test. Since variables were normally distributed, differences between tests at Exh1 were determined using one-way repeated measures analysis of variance (ANOVA). The Mauchly's test of sphericity was run before the ANOVA and in the case of violation of the sphericity assumption the degrees of freedom were adjusted according to the Huynh and Feldt test. Pairwise comparisons at specific time points were performed with Student's paired *t*-tests and adjusted for multiple comparisons with the Holm–Bonferroni method. Since no significant differences were observed at exhaustion between the four tests in severe hypoxia (P_IO_2 = 73 mmHg), these four tests were averaged to obtain a representative value for exhaustion at a P_IO_2 of 73 mmHg. The same procedure was used to test for differences between the four IE tests ending in normoxia (Exh3). Similar results were obtained in the four tests at exhaustion in normoxia (Exh3) and hence, the values obtained in these four tests were also averaged to generate a single value representing normoxia. These two averages were compared with Student's paired *t*-tests. The effect of increasing P_IO_2 at exhaustion on all dependent variables was assessed using a two-way ANOVA for repeated measures with two factors: breathing gas (two levels: pre- vs. post-switch to the new breathing gas) and P_IO_2 (four levels), followed by pairwise comparisons using Student's paired *t*-tests adjusted for multiple comparisons with the Holm–Bonferroni method. The relationships between changes in P_IO_2 and the changes in the dependent variables were tested using linear regression analysis. To compare the first 10 s of the transition between the first and the second transition, an average value for the four conditions of each transition was calculated. This generated a single value per subject for the first and another unique value per subject for the second transition. The two transitions were compared with a paired Student's *t*-test. Values are reported as the mean ± standard deviation (unless otherwise stated). $P \leq 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS v.15.0 for Windows (SPSS Inc., Chicago, IL) and Excel 2011 (Microsoft, Redmond, WA, USA).

RESULTS

Maximal Exercise in Severe Acute Hypoxia (P_IO_2 = 73 mmHg) and Normoxia (P_IO_2 = 142 mmHg)

As shown in **Table 1**, SpO_2, power output at exhaustion (Wmax), VO_2peak, pulmonary ventilation at exhaustion (V_E), respiratory rate (RR), heart rate at exhaustion (HR), end-tidal O_2 pressure ($P_{ET}O_2$), end-tidal CO_2 pressure ($P_{ET}CO_2$), and carbon dioxide production (VCO_2) were lower during the last 30 s of exercise in severe hypoxia than in normoxia, while the respiratory exchange ratio (RER) was higher in hypoxia than in normoxia (all $P \leq 0.05$).

TABLE 1 | Ergospirometric and electromyographic responses during the last 30 s of the incremental exercise to exhaustion in normoxia (P_IO_2 ≈ 142 mmHg) and severe hypoxia (P_IO_2 ≈ 73 mmHg).

	Hypoxia (P_IO_2= 73 mmHg)	Normoxia	*P*
F_IO_2 (%)	10.8 ± 0.07	20.8 ± 0.04	< 0.001
SpO_2 (%)	63.8 ± 5.7	92.8 ± 3.1	< 0.001
Wmax (W)	170.5 ± 17.9	213 ± 19.7	< 0.001
VO_2peak (L.min^{-1})	2.28 ± 0.19	3.44 ± 0.43	< 0.001
V_E (L.min^{-1})	115.2 ± 18.6	124.8 ± 15.6	< 0.001
RR (breaths.min^{-1})	50.6 ± 7.0	55.9 ± 7.1	< 0.001
HR (beats.min^{-1})	179.0 ± 8.4	184.8 ± 5.2	< 0.001
$P_{ET}O_2$ (mmHg)	51.3 ± 2.3	108.2 ± 7.4	< 0.001
$P_{ET}CO_2$ (mmHg)	28.1 ± 2.5	30.8 ± 3.1	< 0.001
RER	1.34 ± 0.13	1.05 ± 0.06	< 0.001
VCO_2 (L.min^{-1})	3.06 ± 0.36	3.55 ± 0.41	< 0.001
RPM	71.9 ± 4.1	68.4 ± 4.2	0.08
VM RMSraw (μV)	111.2 ± 38.6	128.3 ± 42.4	< 0.01
VL RMSraw (μV)	97.5 ± 30.8	110.4 ± 28.0	< 0.01
Average RMSraw (μV)	104.4 ± 29.0	119.4 ± 28.8	< 0.005
VM RMSNz (A.U.)	178.1 ± 35.2	209.2 ± 58.4	< 0.05
VL RMSNz (A.U.)	173.3 ± 35.4	200.0 ± 49.1	< 0.005
Average RMSNz (A.U.)	175.4 ± 31.2	204.6 ± 50.3	< 0.01
VM TAINz (A.U.)	111.5 ± 33.5	138.7 ± 47.8	< 0.005
VL TAINz (A.U.)	97.3 ± 21.0	117.4 ± 22.7	< 0.001
Average TAINz (A.U.)	102.9 ± 25.5	126.7 ± 30.9	< 0.001
VM MPF (Hz)	89.8 ± 16.9	85.2 ± 16.6	< 0.001
VL MPF (Hz)	89.6 ± 16.5	85.5 ± 17.1	< 0.001
Average MPF (Hz)	89.7 ± 16.7	85.4 ± 16.9	< 0.001
VM MdPF (Hz)	71.3 ± 12.1	69.2 ± 11.8	0.06
VL MdPF (Hz)	70.6 ± 12.1	68.9 ± 12.2	0.06
Average MdPF (Hz)	70.9 ± 12.1	69.0 ± 12.0	0.06
VM Burst (ms)	305.4 ± 51.5	334.3 ± 34.8	< 0.05
VL Burst (ms)	283.0 ± 34.5	306.2 ± 26.7	< 0.05
Average Burst (ms)	294.2 ± 42.1	320.2 ± 29.6	< 0.05

F_IO_2, inspiratory oxygen fraction; SpO_2, hemoglobin saturation in capillary blood measured by pulse oximetry; Wmax, power output at exhaustion; VO_2, oxygen consumption; V_E, pulmonary ventilation; RR, respiratory rate; HR, heart rate; $P_{ET}O_2$, end-tidal O_2 pressure; $P_{ET}CO_2$, end-tidal CO_2 pressure; RER, respiratory exchange ratio; VCO_2, CO_2 production; RPM, revolutions per minute; VL, vastus lateralis; VM, vastus medialis; RMSraw, raw root mean square; RMSNz, normalized root mean square; TAINz, normalized total activation index (arbitrary units, A.U.); MPF, mean power frequency; MdPF, median power frequency; Burst, contraction burst duration. n = 10.

Muscle activation, as reflected by VM and VL raw and normalized RMS, total activation index and contraction burst duration was 8–20% lower in hypoxia than normoxia ($P < 0.05$) (**Table 1**). In contrast, MPF was 5% lower in normoxia than hypoxia ($P < 0.001$) and a similar trend was observed for MdPF (**Table 1**).

Effect of Increased P_IO_2 on cardiorespiratory and EMG Variables

Increased P_IO_2 allowed for the continuation of exercise during 41.9 ± 19.8, 60.7 ± 30.2, 72.9 ± 52.0, and 170.5 ± 70.8 s for the transition from a P_IO_2 of 73 mmHg to placebo, 82, 92, and 99 mmHg, respectively, (all $P < 0.05$, compared to the end exercise in severe acute hypoxia). There was a linear relationship between the duration of the new oxygenation phases and the increase of P_IO_2 (time (s) = 35.1+ 4.86· ΔP_IO_2; $R^2 = 0.955$, $P < 0.001$, $n = 8$), where ΔP_IO_2 represents the increase in P_IO_2 in mmHg (**Figure 3A**). A similar relationship was obtained between endurance time and the estimated improvement in SaO_2 (time (s) = 21.1+ 9.17· ΔS_aO_2; $R^2 = 0.973$, $P < 0.001$, $n = 8$) (**Figure 3B**).

Compared to the mean values observed during the last 10 s of exercise in severe hypoxia (P_IO_2 = 73 mmHg), $P_{ET}O_2$, and VO_2 were increased, and RER reduced during the first 10 s following the increase in oxygenation (**Tables 2** and **3**). SpO_2 was only significantly increased in transitions to normoxia (**Table 3**), in part due to the slow response time of the pulse oximeter. These effects were more accentuated the greater the difference in P_IO_2 between the hypoxic and the increased P_IO_2 condition.

Transition from Severe Hypoxia (P_IO_2 of 73 mmHg) to Higher Levels of P_IO_2

VL and VM RMSraw, RMSNz and TAINz were all enhanced by increasing the P_IO_2 at exhaustion (ANOVA main breathing gas effect $P < 0.05$) (**Table 2**). VM and VL RMSraw, as well as the VM-VL average RMSraw, were increased by 5–10% when the P_IO_2 was raised from 73 to 92, or 99 mmHg (**Table 2**). VL RMSraw and the VM-VL average RMSraw were also increased when the P_IO_2 was raised from 73 to 142 mmHg (**Table 3**). MPF and MdPF remained at the same level with the increase of P_IO_2.

Transition to Normoxia

As depicted in **Table 3**, increasing P_IO_2 from different hypoxia conditions to normoxia was also associated to increased VM and VL RMSraw and RMSNz, as well at VM TAINz and VM-VL Average TAINz (ANOVA breathing gas main effect $P < 0.05$) (**Table 3**). When the data from the two conditions with greater levels of hypoxia (P_IO_2 of 73 and 82 mmHg) were averaged, increasing P_IO_2 at exhaustion to normoxia significantly increased MA (RMSraw and RMSNz) and the normalized TAI ($P < 0.05$). However, this was not the case when the data from the less hypoxic conditions (P_IO_2 of 92 and 99 mmHg) were averaged, for which the transition to higher P_IO_2 did not result in significantly greater muscle activation. In general, MPF and MdPF remained at the same level or changed slightly with the transition to an increased P_IO_2.

We also analyzed the 10 s comprised between the 5th and the 15th second after the start of the transition and compared these 10 s with the last 10 s of the preceding exercise phase. The results of this analysis were essentially similar to those described above, i.e., increasing P_IO_2 at exhaustion resulted in increased MA (RMSraw and RMSNz), particularly when fatigue occurred at high levels of hypoxia (P_IO_2 of 73 and 82 mmHg).

In general, the pedaling rate was augmented with increased oxygenation at the transition from different levels of hypoxia to normoxia, and consequently, the duration of the contraction bursts was reduced (**Table 3**). At the same time, the start of the

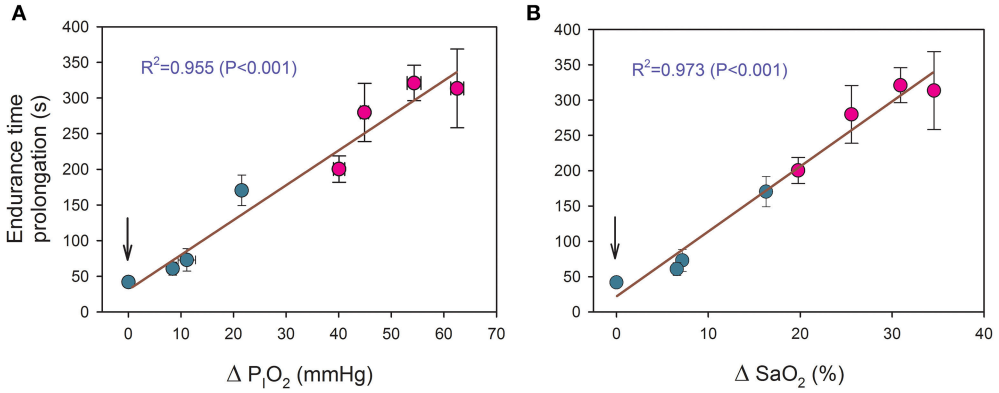

FIGURE 3 | Relationship between the duration of new oxygenation phases with: (A) the increase of P_IO_2 (ΔP_IO_2) and (B) the estimated improvement in arterial saturation (ΔSaO_2). Green circles: transitions from severe hypoxia (P_IO_2 = 73 mmHg) to placebo (vertical arrow) and moderate hypoxia; red circles: transitions from different levels of hypoxia (P_IO_2 of 73, 82, 92, and 99 mmHg) to normoxia. Each point corresponds to the mean of 10 subjects.

contraction bursts occurred slightly earlier with an increase in oxygenation from a P_IO_2 of 73 mmHg to normoxia.

The First Transition Compared with the Second Transition

In the first transition, the P_IO_2 was increased from severe hypoxia (P_IO_2 = 73 mmHg) to less hypoxic levels, while during the second transition the P_IO_2 was increased from different levels of hypoxia to normoxia. We calculated a mean value for the four P_IO_2 conditions of the first transition and compared it with the mean value calculated using the four conditions of the second transition, including in the analysis only the breath-by-breath data collected during the first 10 s of each transition. The mean P_IO_2 during the first and second transition was 84.3 ± 2.1, and 137.5 ± 3.0 mmHg, respectively, ($P < 0.001$); while SpO_2 was 64.1 ± 4.8 and 72.0 ± 4.7%, respectively, ($P < 0.001$). The mean exercise intensity at which the first and second transitions occurred was 170.5 ± 17.9 and 173.5 ± 16.3 W ($P = 0.08$). The mean response of heart rate, pulmonary ventilation, respiratory rate and tidal volume were similar in both transitions (**Figures 4A–D**, respectively). In contrast, the $P_{ET}CO_2$, $P_{ET}O_2$, VO_2, and VCO_2 were higher during the second transition (**Figures 4E–H**, respectively).

Muscle activation was 6% higher during second compared to the first transition, as reflected by the VM, VL, and VM-VL average RMSraw values ($P < 0.05$) (**Figure 5A**). Similar results were obtained for the VM and VM-VL average RMSNz, which were 8 and 7% higher during the second compared to the first transition, respectively ($P < 0.05$) (**Figure 5B**). The VM, VL, and VM-VL average TAINz values were 8-10% higher during the second than the first transition ($P < 0.05$) (**Figure 5C**). VM, VL, and VM-VL average mean and median power frequencies were 4–6% lower during the second than the first transition ($P < 0.001$) (**Figures 5D** and **E**). The start of the burst occurred slightly earlier in the pedaling cycle during the second compared to the first transition for the VM and VM-VL average values, respectively, ($P < 0.05$) (**Figure 5F**). The duration of the burst and the mean pedaling rates were similar during both transitions ($P > 0.56$) (**Figures 5G** and **H**).

Importance of the Magnitude of the Change in P_IO_2 and the Pre-existing Level of Hypoxia on the Response to an Increase in P_IO_2

As reflected in **Figure 6**, the changes of $P_{ET}O_2$, VO_2, the duration of the bursts and pedaling rate (PR) were linearly related to the increase in P_IO_2 as shown in the equations:

$$\Delta VO_2 = 0.0277 \cdot \Delta P_IO_2 - 0.0514 (R^2 = 0.990; P < 0.001; n = 8); \quad \text{Equation 1, (Figure 6A)}$$

$$\Delta P_{ET}O_2 = 0.654 \cdot \Delta P_IO_2 - 0.852 (R^2 = 0.997; P < 0.001; n = 8); \quad \text{Equation 2, (Figure 6B)}$$

$$\Delta BD = 16.33 - 1.127 \cdot \Delta P_IO_2 (R^2 = 0.941; P < 0.001; n = 8); \quad \text{Equation 3, (Figure 6E)}$$

$$\Delta PR = 0.082 - 1.122 \cdot \Delta P_IO_2 (R^2 = 0.917; P < 0.001; n = 8); \quad \text{Equation 4, (Figure 6F)}$$

Where ΔVO_2 is expressed in $L \cdot min^{-1}$; $\Delta P_{ET}O_2$ and ΔP_IO_2 in mmHg; BD in ms, and PR in rpm.

The VM-VL average RMSraw was linearly related to the increase in P_IO_2, but only in the transitions from a P_IO_2 of 73 mmHg to a higher P_IO_2 [Δ RMSraw (μV) = 1.945 + 0.449 · ΔP_IO_2 ($R^2 = 0.915$; $P < 0.05$, $n = 4$)] (**Figure 6C**). This relationship was lost after normalization of the RMS (**Figure 6D**).

Placebo Effects

In the placebo transition, subjects believed that they were receiving normoxia upon exhaustion in severe hypoxia; however, they were maintained at the same level of hypoxia. No significant changes were observed in MA (RMSNz and TAINz) as a consequence of this placebo treatment (**Table 2**).

TABLE 2 | Cardiorespiratory responses during the last 10 s of an incremental exercise to exhaustion in severe hypoxia (P_IO_2 = 73 mmHg) and during the first 10 s of oxygenation with different gas mixtures.

	Exhaustion P_IO_2 = 73 mmHg	Start of P_IO_2 = 99 mmHg	Exhaustion P_IO_2 = 73 mmHg	Start of P_IO_2 = 92 mmHg	Exhaustion P_IO_2 = 73 mmHg	Start of P_IO_2 = 82 mmHg	Exhaustion P_IO_2 = 73 mmHg	Start of P_IO_2 = 73 mmHg
F_IO_2 (%)	10.78 ± 0.10	13.92 ± 0.23^{c}	10.82 ± 0.12	12.45 ± 0.75^{c}	10.78 ± 0.06	12.01 ± 0.25^{c}	10.79 ± 0.08	10.79 ± 0.20¶§‡
SpO_2 (%)	62.4 ± 5.2	63.1 ± 5.5	64.3 ± 5.5	65.1 ± 5.7	63.5 ± 5.8	63.6 ± 5.9	64.7 ± 4.9	64.4 ± 5.7‡
Wmax (W)	172.0 ± 23.5	172.0 ± 23.5	170.0 ± 21.6	170.0 ± 21.6	168.0 ± 16.9	168.0 ± 16.9	172.0 ± 21.5	172.0 ± 21.5
VO_2peak (L.min^{-1})	2.32 ± 0.17	2.81 ± 0.46^{b}	2.23 ± 0.25	2.53 ± 0.34^{a}	2.33 ± 0.15	2.45 ± 0.27^{T}	2.27 ± 0.29	2.29 ± 0.32¶§‡
V_E (L.min^{-1})	118.2 ± 23.7	111.8 ± 20.8	114.8 ± 26.3	116.8 ± 22.6	117.5 ± 16.4	116.4 ± 15.1	114.8 ± 14.7	116.8 ± 15.6
RR (breaths.min^{-1})	51.7 ± 9.2	48.1 ± 7.2	51.3 ± 8.5	50.7 ± 8.2	51.5 ± 8.4	51.1 ± 7.6	51.3 ± 7.0	52.3 ± 7.7
HR (beats.min^{-1})	179.0 ± 10.2	179.3 ± 10.1	180.5 ± 8.2	181.0 ± 7.4	177.3 ± 7.4	177.5 ± 7.2	180.6 ± 7.9	180.8 ± 8.3¶
$P_{ET}O_2$ (mmHg)	51.4 ± 3.0	62.9 ± 5.1^{c}	51.7 ± 3.1	58.1 ± 6.3^{c}	51.4 ± 2.9	56.1 ± 3.9^{c}	51.6 ± 2.0	51.9 ± 2.0¶§‡
$P_{ET}CO_2$ (mmHg)	27.3 ± 2.9	27.3 ± 4.3	28.3 ± 2.9	28.4 ± 2.7	27.4 ± 3.7	27.6 ± 3.0	28.5 ± 2.3	28.2 ± 2.4
RER	1.32 ± 0.17	1.16 ± 0.15^{T}	1.36 ± 0.15	1.28 ± 0.13	1.32 ± 0.15	1.28 ± 0.15	1.36 ± 0.14	1.37 ± 0.17§
VCO_2 (L.min^{-1})	3.06 ± 0.42	3.01 ± 0.44	3.03 ± 0.48	3.12 ± 0.41	3.07 ± 0.29	3.06 ± 0.28	3.08 ± 0.41	3.11 ± 0.39
RPM	63.6 ± 9.4	66.3 ± 10.3	68.5 ± 7.0	71.3 ± 11.4	67.1 ± 9.5	68.2 ± 12.0	71.2 ± 7.8	70.0 ± 9.3
VM RMSraw (μV)	105.9 ± 37.2	119.3 ± 43.5^{c}	106.3 ± 42.6	112.2 ± 38.1^{a}	113.5 ± 42.7	120.6 ± 50.2^{T}	97.2 ± 43.0	99.7 ± 42.6¶‡
VL RMSraw (μV)	108.8 ± 38.4	119.1 ± 39.9^{c}	97.3 ± 48.9	102.0 ± 49.2^{a}	115.8 ± 49.9	114.1 ± 45.1	85.9 ± 35.5	87.4 ± 40.6¶
Average RMSraw (μV)	107.4 ± 29.1	119.2 ± 33.4^{c}	101.8 ± 41.5	107.1 ± 39.1^{b}	114.7 ± 39.2	117.3 ± 40.6	91.5 ± 34.9	93.6 ± 38.0¶‡
VM RMSNz (A.U.)	176.2 ± 48.1	195.8 ± 56.9^{c}	178.0 ± 58.4	191.2 ± 57.2^{a}	180.9 ± 50.4	187.6 ± 41.7	164.8 ± 67.2	168.6 ± 64.2¶
VL RMSNz (A.U.)	182.5 ± 55.5	199.6 ± 52.2^{c}	160.2 ± 38.8	171.1 ± 35.5^{a}	189.6 ± 67.0	185.6 ± 45.4	151.7 ± 49.2	152.3 ± 51.7¶‡
Average RMSNz (A.U.)	179.4 ± 49.8	197.7 ± 52.5^{c}	169.1 ± 47.0	181.2 ± 45.0^{a}	185.2 ± 56.4	186.6 ± 40.2	158.2 ± 56.8	160.5 ± 56.9¶‡
VM TAINz (A.U.)	39.6 ± 17.6	44.5 ± 19.5^{b}	35.6 ± 14.8	38.0 ± 12.5	37.1 ± 14.1	37.6 ± 11.1	33.5 ± 13.3	35.5 ± 14.1¶
VL TAINz (A.U.)	36.8 ± 13.5	41.2 ± 14.4^{c}	30.2 ± 9.7	32.8 ± 7.1	37.1 ± 12.6	35.8 ± 8.7	29.3 ± 8.8	31.6 ± 14.0¶
Average TAINz (A.U.)	38.2 ± 15.4	42.8 ± 16.9^{c}	32.9 ± 11.8	35.4 ± 9.5	37.1 ± 13.2	36.7 ± 9.6	31.4 ± 10.6	33.6 ± 13.3¶
VM MPF (Hz)	96.0 ± 24.8	95.1 ± 24.2	89.2 ± 22.8	91.5 ± 23.9	91.2 ± 17.3	89.8 ± 15.0	84.9 ± 15.5	83.6 ± 13.8
VL MPF (Hz)	97.0 ± 27.0	96.0 ± 27.5	88.9 ± 22.6	90.2 ± 23.2	91.2 ± 17.3	89.5 ± 15.5	84.9 ± 15.9	83.5 ± 14.4
Average MPF (Hz)	96.5 ± 25.9	95.5 ± 25.7	89.1 ± 22.7	90.9 ± 23.5	91.2 ± 17.3	89.6 ± 15.3	84.9 ± 15.7	83.5 ± 14.1
VM MdPF (Hz)	76.3 ± 17.1	76.9 ± 17.1	70.3 ± 15.7	72.7 ± 14.7	70.8 ± 13.2	71.8 ± 11.6	66.9 ± 10.3	66.2 ± 10.5
VL MdPF (Hz)	77.8 ± 19.7	77.6 ± 20.0	69.8 ± 15.9	70.9 ± 15.0	70.7 ± 13.2	71.4 ± 12.0	66.4 ± 10.8	65.6 ± 10.2
Average MdPF (Hz)	77.0 ± 18.3	77.3 ± 18.4	70.0 ± 15.8	71.8 ± 14.7	70.8 ± 13.2	71.6 ± 11.7	66.7 ± 10.5	65.9 ± 10.3§
VM Burst (ms)	361.5 ± 110.1	349.9 ± 85.9	310.7 ± 88.3	305.1 ± 81.2	310.3 ± 62.5	304.9 ± 94.7	309.3 ± 80.7	318.2 ± 74.7
VL Burst (ms)	343.2 ± 91.7	324.8 ± 81.7	297.8 ± 55.6	300.1 ± 70.7	310.2 ± 59.7	303.9 ± 91.5	300.0 ± 63.7	316.5 ± 86.6
Average Burst (ms)	352.4 ± 95.3	337.3 ± 79.0	304.3 ± 71.3	302.6 ± 75.4	310.2 ± 60.8	304.4 ± 93.0	304.7 ± 71.0	317.4 ± 76.5
VM Timing (%)	48.8 ± 3.8	47.6 ± 4.2^{a}	49.4 ± 2.5	49.1 ± 3.2	49.4 ± 2.3	49.3 ± 2.9	47.9 ± 3.9	48.3 ± 4.2‡
VL Timing (%)	50.7 ± 2.1	50.0 ± 2.3	50.2 ± 2.3	49.9 ± 2.5	50.3 ± 2.1	50.3 ± 2.4	49.5 ± 2.9	49.6 ± 3.0
Average Timing (%)	49.7 ± 2.6	48.8 ± 2.7	49.8 ± 2.3	49.5 ± 2.7	49.8 ± 2.2	49.8 ± 2.6	48.7 ± 3.4	49.0 ± 3.5

F_IO_2, inspiratory oxygen fraction; SpO_2, hemoglobin saturation in capillary blood measured by pulse oximetry; Wmax, power output at exhaustion; VO_2, oxygen consumption; V_E, pulmonary ventilation; RR, respiratory rate; HR, heart rate; $P_{ET}O_2$, end-tidal O_2 pressure; $P_{ET}CO_2$, end-tidal CO_2 pressure; RER, respiratory exchange ratio; VCO_2, CO_2 production; RPM, revolutions per minute; VL, vastus lateralis; VM, vastus medialis; RMSraw, raw root mean square; RMSNz, normalized root mean square; TAINz: normalized total activation index (arbitrary units, A.U.); MPF, mean power frequency; MdPF, median power frequency; Burst, contraction burst duration; Timing: start of activation expressed as percentage of total revolution duration. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$; and $^{T}P < 0.1$ (F_IO_2 = 73 mmHg vs. new gas mixture). $^{¶}P < 0.05$ ANOVA breathing gas switch main effect; $^{§}P < 0.05$ ANOVA oxygenation level main effect; $^{‡}P < 0.05$ ANOVA breathing gas switch x oxygenation level interaction; n = 10.

DISCUSSION

This study shows that MA during the last 10–30 s of an IE to exhaustion is lower in SAH than in normoxia, while at exhaustion in moderate hypoxia MA was similar to that observed at exhaustion in normoxia. We have shown that during exercise at different levels of hypoxia, increasing P_IO_2 at exhaustion with normoxic or less hypoxic gas mixtures rapidly relieves fatigue and allows for the continuation of exercise. This effect is accompanied by increased MA only when the level of hypoxia during the exercise eliciting exhaustion was severe (P_IO_2 of 73 mmHg, equivalent to an altitude close to 5200 m) and the P_IO_2 was increased to 92 mmHg or higher and the estimated SaO_2 to 70% or higher. Nevertheless, the close linear relationship between the increase in MA (average of VM and VL RMSraw) and the increase in P_IO_2 (**Figure 6C**) indicates that during exercise in SAH any small increase in P_IO_2 could have a positive effect on muscle activation. This is also supported by the fact that during the first 10 s of the transitions, MA was higher during the second than the first transition, despite the fact

TABLE 3 | Cardiorespiratory responses during the last 10 s of an incremental exercise to exhaustion in different levels of hypoxia (P_IO_2 = 73, 82, 92, and 99 mmHg) and during the first 10 s of oxygenation to normoxia (P_IO_2 = 142 mmHg).

	Exhaustion P_IO_2 = 99 mmHg	Start of Normoxia	Exhaustion P_IO_2 = 92 mmHg	Start of Normoxia	Exhaustion P_IO_2 = 82 mmHg	Start of Normoxia	Exhaustion P_IO_2 = 73 mmHg	Start of Normoxia
F_IO_2 (%)	14.42 ± 0.12	20.26 ± 0.46^{c}	13.41 ± 0.39	19.99 ± 0.53^{c}	11.98 ± 0.21	19.90 ± 0.62^{c}	10.91 ± 0.44	20.02 ± 0.74^{c¶§‡}
SpO_2 (%)	78.2 ± 4.1	80.2 ± 5.3^{a}	70.4 ± 6.7	73.4 ± 6.8^{c}	67.6 ± 4.5	68.2 ± 4.2^{b}	64.7 ± 4.8	66.3 ± 6.20^{a¶§‡}
Wmax (W)	180.0 ± 21.1	180.0 ± 21.1	172.0 ± 19.3	172.0 ± 19.3	170.0 ± 17.0	170.0 ± 17.0	172.0 ± 21.5	172.0 ± 21.5
VO_2peak (L.min^{-1})	2.93 ± 0.25	3.89 ± 0.68^{b}	2.87 ± 0.28	4.15 ± 0.45^{c}	2.48 ± 0.32	3.99 ± 0.52^{c}	2.37 ± 0.30	4.05 ± 0.59^{c¶§‡}
V_E (L.min^{-1})	119.9 ± 18.1	116.1 ± 25.6	118.6 ± 19.0	117.3 ± 16.7	110.8 ± 24.5	109.5 ± 17.8	117.6 ± 17.5	113.6 ± 21.2
RR (breaths.min^{-1})	53.5 ± 7.1	51.2 ± 5.7	53.2 ± 7.0	52.6 ± 6.0	49.6 ± 8.6	49.5 ± 5.6	52.7 ± 7.2	52.2 ± 7.7
HR (beats.min^{-1})	182.2 ± 7.9	182.1 ± 8.7	182.7 ± 6.2	182.9 ± 6.2	178.6 ± 7.0	178.5 ± 6.5	180.9 ± 7.9	180.5 ± 7.9
$P_{ET}O_2$ (mmHg)	71.3 ± 2.6	96.4 ± 9.9^{c}	64.4 ± 3.1	90.6 ± 13.4^{c}	56.7 ± 3.4	91.4 ± 12.2^{c}	51.8 ± 2.1	92.3 ± 11.5^{c¶§‡}
$P_{ET}CO_2$ (mmHg)	29.0 ± 2.9	30.5 ± 3.0^{a}	29.0 ± 2.9	30.1 ± 2.5^{a}	28.7 ± 3.6	29.5 ± 3.5^{a}	28.3 ± 2.4	30.2 ± 2.7^{a}
RER	1.13 ± 0.08	0.92 ± 0.10^{c}	1.12 ± 0.08	0.87 ± 0.12^{c}	1.21 ± 0.11	0.84 ± 0.10^{c}	1.35 ± 0.14	0.90 ± 0.15^{c¶§‡}
VCO_2 (L.min^{-1})	3.28 ± 0.28	3.29 ± 0.51	3.21 ± 0.34	3.25 ± 0.29	3.00 ± 0.47	3.02 ± 0.37	3.13 ± 0.40	3.16 ± 0.41§
RPM	61.7 ± 9.0	67.0 ± 12.5	63.8 ± 11.4	69.5 ± 10.2^{b}	61.9 ± 11.3	68.1 ± 9.4^{T}	58.3 ± 12.6	65.6 ± 13.9^{T¶}
VM RMSraw (μV)	116.9 ± 45.3	122.7 ± 46.6	120.2 ± 42.5	119.8 ± 39.2	120.1 ± 49.4	125.3 ± 47.6	102.6 ± 47.0	108.4 ± 44.3
VL RMSraw (μV)	116.5 ± 38.1	123.6 ± 43.3	107.3 ± 52.5	110.8 ± 52.6	118.4 ± 50.2	122.7 ± 52.9	87.2 ± 36.1	94.5 ± 37.0^{b¶}
Average RMSraw (μV)	116.7 ± 32.5	123.2 ± 35.9	113.8 ± 42.6	115.3 ± 40.3	119.3 ± 41.6	124.0 ± 43.1	94.9 ± 37.5	101.5 ± 36.3^{a¶}
VM RMSNz (A.U.)	182.1 ± 53.9	191.7 ± 57.8	204.3 ± 70.8	205.8 ± 68.7	189.5 ± 57.3	197.9 ± 51.7	172.9 ± 70.7	184.7 ± 77.1¶
VL RMSNz (A.U.)	195.4 ± 52.6	206.8 ± 66.6	177.8 ± 41.3	183.4 ± 43.3	194.5 ± 70.0	199.4 ± 57.5	155.9 ± 55.7	167.3 ± 53.4^{a¶}
Average RMSNz (A.U.)	188.7 ± 43.9	199.2 ± 52.7	191.1 ± 53.8	194.6 ± 50.8	192.0 ± 61.4	198.6 ± 51.6	164.4 ± 62.0	176.0 ± 63.9¶
VM TAINz (A.U.)	39.9 ± 15.7	43.6 ± 16.2^{T}	42.8 ± 19.2	40.6 ± 11.2	42.1 ± 17.0	42.6 ± 14.5	36.1 ± 14.4	38.0 ± 16.4¶
VL TAINz (A.U.)	43.2 ± 19.3	42.4 ± 14.4	34.1 ± 9.0	35.3 ± 7.2	40.5 ± 15.2	40.9 ± 12.8	32.5 ± 13.8	34.1 ± 15.6
Average TAINz (A.U.)	41.5 ± 17.1	43.0 ± 15.1	38.4 ± 13.4	37.9 ± 8.4	41.3 ± 15.9	41.7 ± 13.5	34.3 ± 13.5	36.0 ± 15.5¶
VM MPF (Hz)	87.4 ± 20.1	84.9 ± 20.6	84.7 ± 20.2	86.0 ± 21.0	88.5 ± 14.6	87.0 ± 16.6	81.8 ± 15.2	81.7 ± 16.4
VL MPF (Hz)	71.4 ± 15.8	85.3 ± 21.9^{c}	85.7 ± 19.4	85.6 ± 21.8	88.1 ± 15.0	86.3 ± 16.9	81.9 ± 15.5	82.1 ± 16.1¶‡
Average MPF (Hz)	79.4 ± 17.8	85.1 ± 21.2^{b}	85.2 ± 19.8	85.8 ± 21.4	88.3 ± 14.8	86.6 ± 16.7	81.8 ± 15.3	81.9 ± 16.2‡
VM MdPF (Hz)	70.9 ± 16.2	69.9 ± 16.2	67.5 ± 13.6	68.7 ± 15.2	72.1 ± 11.8	70.3 ± 12.2	65.0 ± 10.6	66.1 ± 13.2
VL MdPF (Hz)	71.4 ± 15.8	68.8 ± 16.6^{a}	67.9 ± 13.6	68.1 ± 16.2	71.4 ± 12.1	69.2 ± 12.7	65.5 ± 11.0	65.9 ± 12.5
Average MdPF (Hz)	71.1 ± 16.0	69.3 ± 16.4	67.7 ± 13.6	68.4 ± 15.6	71.8 ± 11.8	69.8 ± 12.3	65.3 ± 10.8	66.0 ± 12.8
VM Burst (ms)	375.6 ± 127.2	363.0 ± 130.0	377.9 ± 196.2	317.6 ± 83.8	372.8 ± 102.7	322.0 ± 73.8^{a}	367.5 ± 85.1	319.2 ± 72.1^{a¶}
VL Burst (ms)	381.7 ± 120.6	321.3 ± 83.7^{a}	343.6 ± 109.4	309.1 ± 73.1^{T}	366.7 ± 107.3	315.0 ± 72.4^{T}	371.4 ± 97.1	309.3 ± 60.0^{a¶}
Average Burst (ms)	378.6 ± 113.0	342.2 ± 92.2^{T}	360.8 ± 150.4	313.3 ± 77.6	369.8 ± 104.6	318.5 ± 58.9^{a}	369.5 ± 88.6	314.2 ± 63.9^{a¶}
VM Timing (%)	49.1 ± 3.4	46.3 ± 7.7	48.2 ± 3.5	48.1 ± 4.4	48.6 ± 3.0	47.8 ± 3.6	49.4 ± 3.4	47.4 ± 4.3^{a¶}
VL Timing (%)	49.5 ± 3.6	50.2 ± 2.2	49.4 ± 2.5	49.7 ± 2.1	50.4 ± 1.6	49.4 ± 2.4	50.5 ± 2.3	49.4 ± 2.8
Average Timing (%)	49.3 ± 3.0	48.3 ± 4.2	48.8 ± 2.7	48.9 ± 3.0	49.5 ± 2.1	48.6 ± 2.8^{T}	50.0 ± 2.8	48.4 ± 3.4^{a¶}

F_IO_2, inspiratory oxygen fraction; SpO_2, hemoglobin saturation in capillary blood measured by pulse oximetry; Wmax, power output at exhaustion; VO_2, oxygen consumption; V_E, pulmonary ventilation; RR, respiratory rate; HR, heart rate; $P_{ET}O_2$, end-tidal O_2 pressure; $P_{ET}CO_2$, end-tidal CO_2 pressure; RER, respiratory exchange ratio; VCO_2,CO_2 production; RPM, revolutions per minute; VL, vastus lateralis; VM, vastus medialis; RMSraw, raw root mean square; RMSNz, normalized root mean square; TAINz: normalized total activation index (arbitrary units, A.U.); MPF, mean power frequency; MdPF, median power frequency; Burst: contraction burst duration; Timing, start of activation expressed as percentage of total revolution duration. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$; and $^{T}P < 0.1$ (F_IO_2 = 73 mmHg vs. new gas mixture). $^{¶}P < 0.05$ ANOVA breathing gas switch main effect; $^{§}P < 0.05$ ANOVA oxygenation level main effect; $^{‡}P < 0.05$ ANOVA breathing gas switch x oxygenation level interaction: n = 10.

that both transitions occurred at comparable exercise intensities. Moreover, our investigation has also demonstrated that an increase in MA after the increase of P_IO_2 at fatigue in hypoxia is not indispensable for the ergogenic effects elicited by the increase of P_IO_2. Collectively, our results suggest that severe hypoxia depresses the capacity of the central nervous system to activate the musculature during whole-body exercise to exhaustion, by a mechanism that can be swiftly reversed by increasing the P_IO_2.

Severe Hypoxia Reduces the Level of Muscle Activation Attainable during Incremental Exercise to Exhaustion

In support of a central predominance of task failure mechanisms is the rapid relief of fatigue with the increase of P_IO_2, e.g., when subjects at exhaustion are asked to continue the exercise once the hypoxic gas mixture they are breathing is swiftly switched to normoxic room air (Calbet et al., 2003a) or hyperoxic gas (Amann et al., 2007). This concurs with the demonstration of a

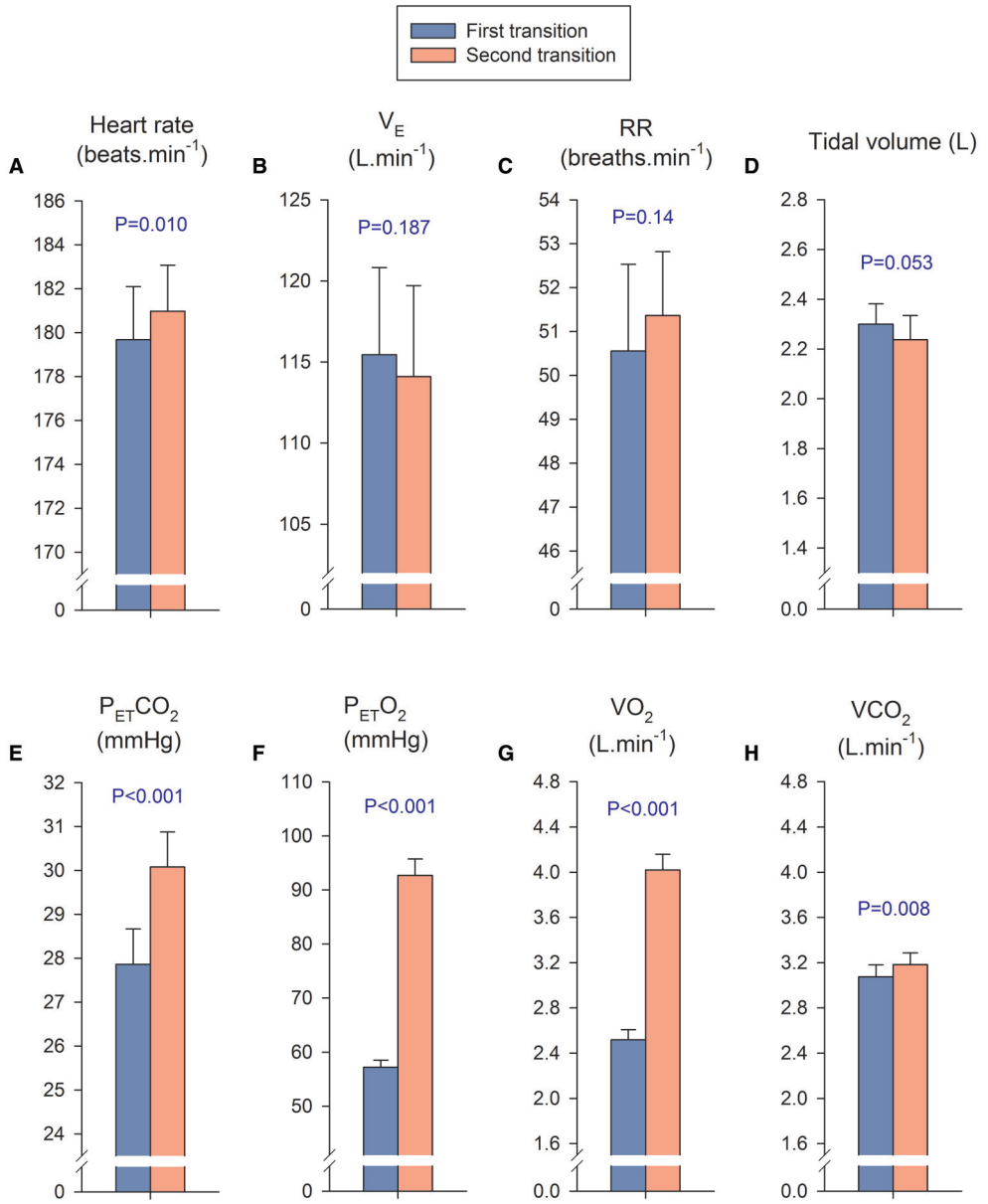

FIGURE 4 | Cardiorespiratory variables during the first and second transition. In the first transition, the P_IO_2 was increased from severe hypoxia (P_IO_2 = 73 mmHg) to less hypoxic levels, while during the second transition the P_IO_2 was increased from different levels of hypoxia to normoxia. We calculated a mean value for the four P_IO_2 conditions of the first transition (blue bars) and compared it with the mean value of the four conditions of the second transition (orange bars), including in the analysis only the breath-by-breath data collected during the first 10 s of each transition. The mean response of the first transition was compared with the mean response of the second transition with a Student's paired *t*- test. It is important to remark that in both transitions the absolute exercise intensity was similar (170–173 W), however there is a remarkable difference in VO_2 which is explained by the massive passage of O_2 from the alveoli to the lung capillaries, driven by the much higher $P_{ET}O_2$ during the second transition. This massive diffusion of O_2 is facilitated by the low SaO_2 of the hemoglobin arriving to the lung capillaries at maximal exercise in hypoxia (Calbet et al., 2016). **(A)** Heart rate; **(B)** B pulmonary ventilation (V_E); **(C)** respiratory rate (RR); **(D)** tidal volumen; **(E)** end-tidal CO_2 pressure ($P_{ET}CO_2$); **(F)** end-tidal O_2 pressure; ($P_{ET}O_2$), **(G)** Oxygen uptake (VO_2); **(H)** CO_2 production (VCO_2). Each bar corresponds to the mean of 10 subjects; error bars represent the standard error of the mean.

greater functional reserve at task failure in SAH than in normoxia (Amann et al., 2007; Calbet et al., 2015a; Morales-Alamo et al., 2015; Torres-Peralta et al., 2016). However, increased P_IO_2 does not relieve fatigue when administered at exhaustion during whole-body exercise in moderate hypoxia (F_IO_2 = 0.15, equivalent to 2700 m above sea level) (Amann et al., 2007) or during exercise recruiting a small muscle mass in severe hypoxia (Calbet and Lundby, 2009).

It has been reported that a greater level of supraspinal fatigue occurs at task failure during whole-body (Goodall et al., 2010)

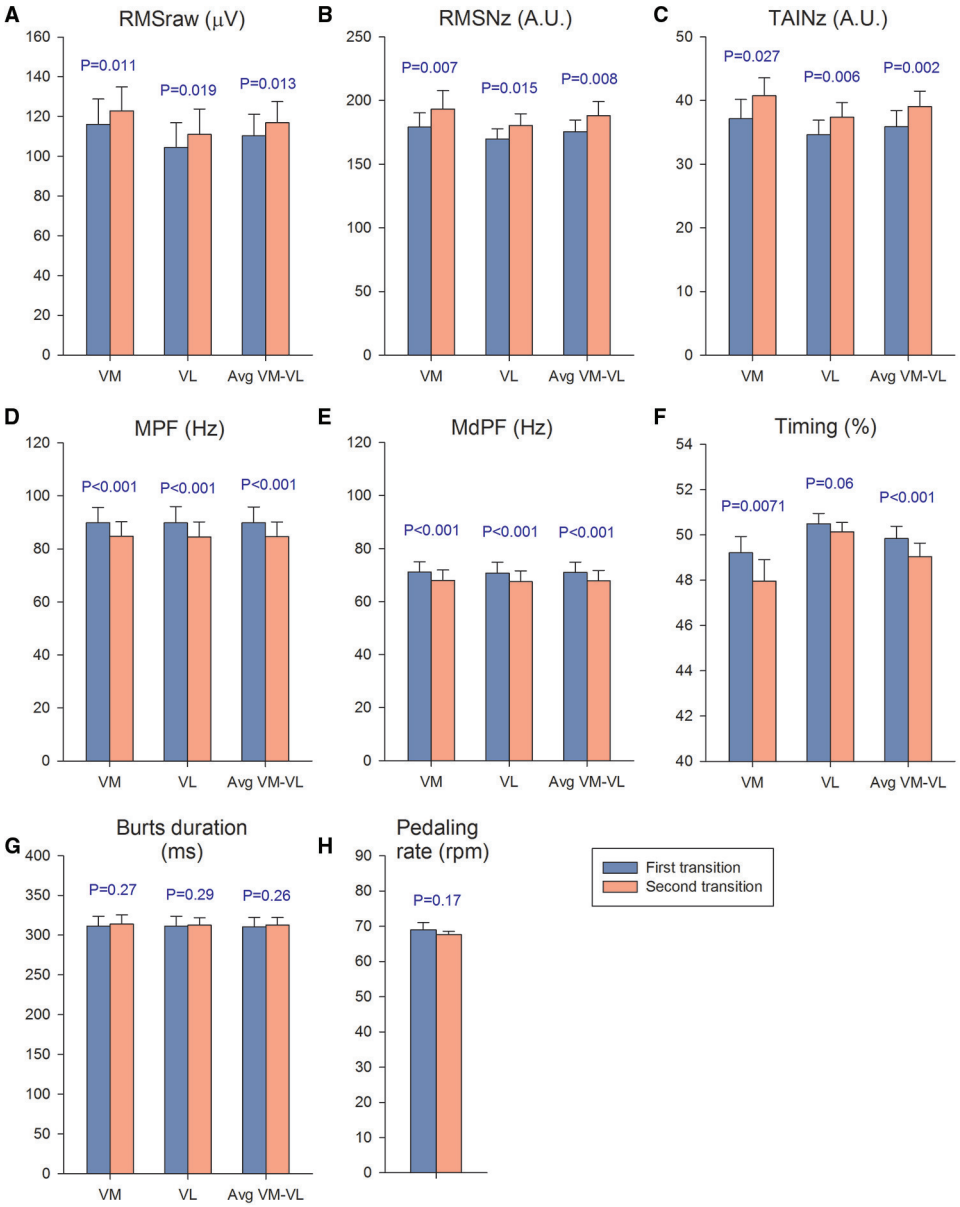

FIGURE 5 | Muscle activation and pedaling rate during the first and second transitions. We calculated a mean value for the four P_IO_2 conditions of the first transition (blue bars) and compared it with the mean value calculated using the four conditions of the second transition (orange bars). The mean response of the first transition was compared with the mean response of the second transition with a Student's paired *t*-test. VM, *vastus medialis*; VL, *vastus lateralis*; **(A)** RMSraw, raw root mean square; **(B)** RMSNz, normalized root mean square; **(C)** TAINz, normalized total activation index; **(D)** MPF, mean power frequency; **(E)** MdPF, median power frequency; **(F)** Timing, start of activation expressed as percentage of total revolution duration; **(G)** burts duration; **(H)** pedaling rate. Each bar corresponds to the mean of 10 subjects; error bars represent the standard error of the mean.

and knee-extension exercise (Goodall et al., 2010) in hypoxia than in normoxia. This effect is more accentuated with increased severity of hypoxia (Goodall et al., 2010). Nevertheless, in contrast to our observations, quadriceps MA (EMG_{RMS}) declined during repeated isometric muscle contractions (60% of the maximal voluntary contraction, 5 s/5 s contraction/recovery) to similar levels in severe hypoxia ($F_IO_2 = 0.10$) and in normoxia (Goodall et al., 2010). A crucial difference between whole-body and small muscle mass (knee extension) exercise in hypoxia is that for a given P_IO_2, pulmonary gas exchange is more perturbed during whole-body than small muscle mass exercise, as reflected by the larger alveolo-arterial O_2 difference ($A\text{-}aDO_2$) observed during whole-body compared to small muscle mass exercise (Calbet et al., 2009b). The larger $A\text{-}aDO_2$ combined with a greater right-shift of the ODC during whole-body exercise in severe hypoxia causes more desaturation for a given PaO_2

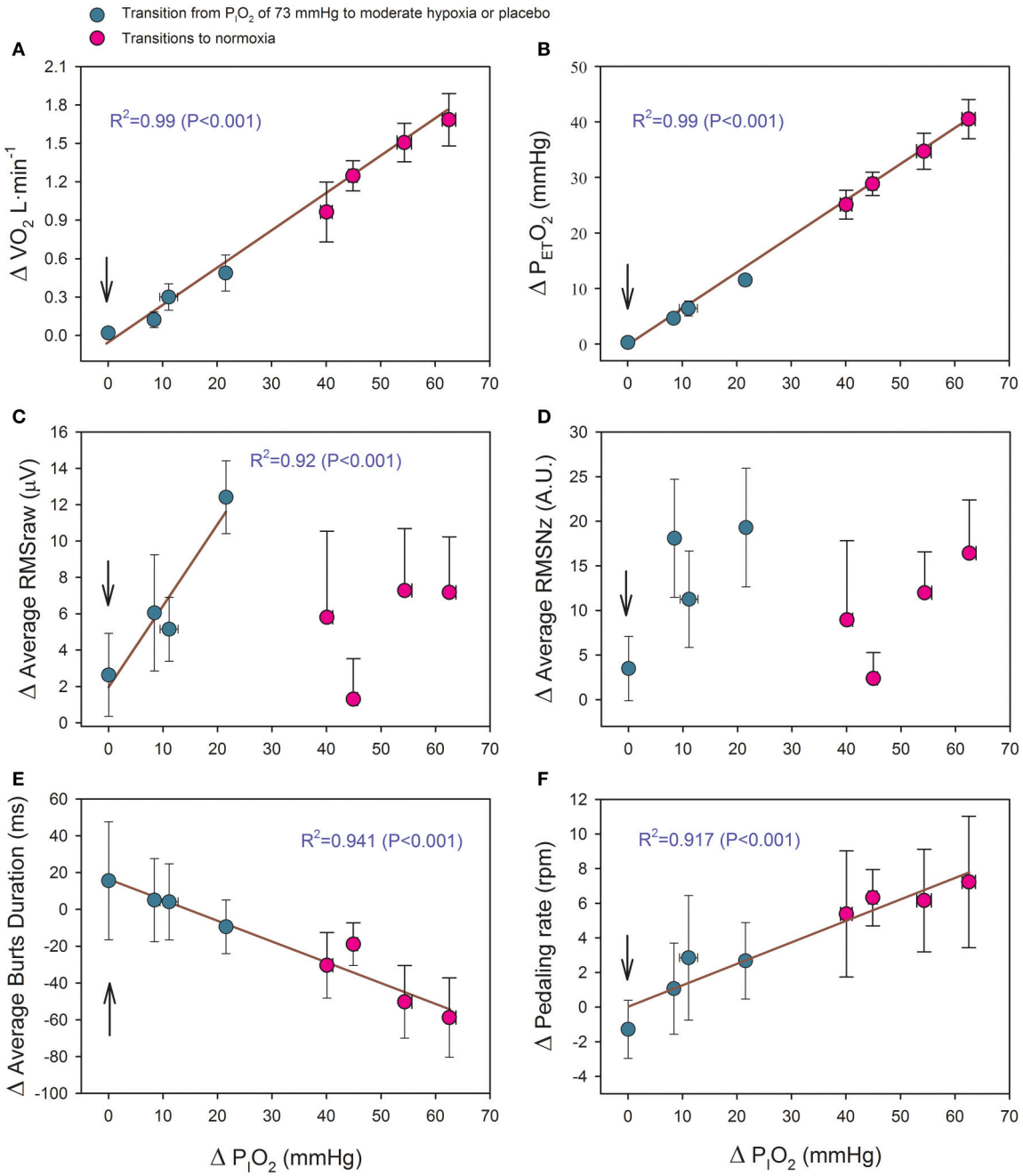

FIGURE 6 | Relationship between the magnitude of the P_IO_2 change (in mmHg) in transition from hypoxia to a higher P_IO_2 and the increases of: (A) oxygen uptake (VO_2), (B) end-tidal O_2 pressure ($P_{ET}O_2$), (C) average raw root mean square (RMSraw) of *vastus medialis* and *vastus lateralis*, (D) average normalized root mean square (RMSNz) of *vastus medialis* and *vastus lateralis*, (E) average burst duration of *vastus medialis* and *vastus lateralis*, and (E) pedaling rate. The vertical arrow indicates the placebo condition. Green circles: transitions from severe hypoxia (P_IO_2 = 73 mmHg) to placebo (vertical arrow) and moderate hypoxia; red circles: transitions from different levels of hypoxia (P_IO_2 of 73, 82, 92, and 99 mmHg) to normoxia. Each point corresponds to the mean of 10 subjects; error bars represent the standard error of the mean.

during whole-body than small muscle exercise in severe hypoxia (Calbet et al., 2009b). Consequently, with an F_IO_2 close to 0.10, SpO_2 at exhaustion was 78% during knee extension exercise in a study by Goodall et al. (2010) and 63% in the current investigation (Calbet et al., 2015a). In the present experiments, SpO_2 was 79% at exhaustion when the P_IO_2 was 99 mmHg, a level of hypoxemia for which acute oxygenation at exhaustion did not enhance muscle activation. Although no definitive

conclusion on which variable, PaO_2 or SaO_2, plays a more important role in the reduction of MA during exercise in severe hypoxia, our data combined with those of Goodall et al. (2010) indicate that MA is lower at exhaustion in hypoxia than in normoxia when the levels of SaO_2 fall below ∼70%, regardless of P_IO_2.

Mechanisms by Which Hypoxia Could Reduce Muscle Activation Close to Exhaustion

Hypothetically, hypoxia could attenuate MA through two main mechanisms. Severe hypoxia could trigger inhibitory feedback at spinal and supraspinal levels reducing the discharge rate of spinal motoneurons compared to normoxia. Alternatively, severe hypoxia could limit or reduce the recruitment of high-threshold motor units. Regarding the first mechanism, animal studies have shown that levels of PaO_2 similar to those observed in this investigation at exhaustion in SAH (Calbet et al., 2015a) increase the baseline discharge frequency of group III and especially of group IV muscle afferents in resting cats (Hill et al., 1992; Lagier-Tessonnier et al., 1993) and rabbits (Arbogast et al., 2000). Increased firing rates by group III/IV muscle afferents may cause reflex inhibition of the α-motoneuron pool (for review see Amann and Kayser, 2009) and, hence, reduced muscle activation. In the present investigation, MPF and MdPF were lower during the second that during the first transition (**Figures 6D** and **F**), coinciding with a greater attenuation of fatigue, likely due to the almost 5 times greater average ΔP_IO_2 during the second than the first transition. This finding does not necessarily indicates a change in motor activation, since MPF and MdPF are poor indices of motor unit recruitment patterns (Farina et al., 2014), and MPF and MdPF may be affected by peripheral factors, as recently demonstrated (Torres-Peralta et al., 2016). In agreement with our interpretation, increased metaboreflex activation during ischemic intermittent isometric muscle knee-extension contractions to exhaustion had no clear inhibitory effects on EMG_{RMS} values (Millet et al., 2009).

Regarding the second mechanism, hypoxia may reduce the oxygenation of the prefrontal, premotor, and motor cortex leading to a mismatch between energy demand and aerobic ATP re-synthesis, which could limit the corticospinal motor drive (Rasmussen et al., 2007; Verges et al., 2012). In agreement with this idea, our subjects developed a lower mean power output during the first 10 s of sprint exercise (30 s Wingate test) in severe hypoxia ($P_IO_2 = 73$ mmHg) than in normoxia, despite the fact that leg VO_2 measured by the direct Fick method, was similar regardless of F_IO_2 (Calbet et al., 2015a). Interestingly, the duration of the contraction bursts was reduced and the start of the contraction bursts slightly advanced in the pedaling cycle with the transition from exhaustion in severe hypoxia to exercise in normoxia (**Table 3**). Likewise, subjects increased their pedaling rate in response to an increase of P_IO_2 at exhaustion. Interestingly, this effect was linearly dependent on the increase in P_IO_2 (**Figure 6F**). The latter implies that enhanced oxygenation (P_aO_2 and/or S_aO_2) at exhaustion swiftly alters the pattern of muscle activation/recruitment, likely allowing for a greater recruitment of faster motor units (Holt et al., 2014). This also points toward a central regulatory mechanism. In contrast, there was no improvement in any variable during the placebo transition, indicating that the level of oxygenation (P_aO_2 and/or S_aO_2) and the central command barely changed. Had the placebo transition reduced the perception of effort, a change would have been expected in the cardiorespiratory response to exercise (Robertson, 1982; Calbet et al., 2015b; Cochrane et al., 2015). Since all subjects believed that they were receiving oxygen-enriched gas at all transitions, included the placebo transition, and no subject was able to guess whether a gas mixture other than normoxia was administered, we can rule out psychological factors as being responsible for the changes in MA elicited by increased P_IO_2. In turn, psychological factors likely explain the ∼42 additional seconds that the subjects were able to exercise during the placebo transition.

Although iEMG increases with increasing angular velocity during concentric contractions (Westing et al., 1991; Amiridis et al., 1996), this factor alone cannot explain the increased MA elicited by increased P_IO_2 in our experiment. In fact, pedaling rate did not increase significantly in the transition from severe hypoxia to moderate hypoxia (i.e., P_IO_2 of 92, and 99 mmHg), while MA was increased.

Limitations

Although the amplitude of the surface EMG signal can provide a useful approximation of the amplitude component of the neural drive to muscle during some controlled conditions including dynamic exercise (Farina et al., 2014; Coelho et al., 2015), it has limitations. For example, the EMG signal is affected by the thickness of the subcutaneous adipose tissue, the spatial resolution is low overrepresenting superficial muscles fibers, may be altered by cross-talk from neighboring muscles, and is affected by the electrical properties of the sarcolemma, which may change during exercise (Farina et al., 2014). None of these factors is expected to change much within the first 10 s of increased P_IO_2 in our experimental conditions because exercise intensity was maintained at the same level during the first 2 min following the change in P_IO_2. The fact that the EMG amplitude of an interference signal is less than that obtained by summing the amplitudes of the individual motor unit action potentials, a phenomenon referred to as amplitude cancelation, also limits the interpretation of our results. Amplitude cancelation increases monotonically as the neural drive to muscle is elevated, affecting mostly the low-threshold motor units (Mottram et al., 2005; Farina et al., 2014). In our experimental conditions, reduced amplitude cancelation during the first 10 s of the transitions from task failure to increased P_IO_2, as a mechanism to explain the increase in EMG amplitude, is also unlikely since increasing P_IO_2 is expected to facilitate the neural drive to the muscles. To circumvent these limitations we have focused on assessing changes during the last 10 s of a given hypoxic condition and the first 10 s of the change to a higher P_IO_2. With such a short period, and given the stability of load at the start of the transition, the metabolic changes in the muscles should have been minuscule. This minimized the potential alteration of the EMG due to modification of the electrical properties of the muscle fibers and

local metabolic factors during the first 10 s of the transition to a higher P_IO_2.

Another limitation of this study is due to the use of finger pulse oximetry rather than the direct assessment of SaO_2, which is less accurate at SaO_2 below 75–85% (Trivedi et al., 1997; Kolb et al., 2004). Another drawback of finger pulse oximetry is related to the slow response time of all pulse oximeters (Trivedi et al., 1997), with the delay being higher for finger than earlobe placements (Trivedi et al., 1997; Hamber et al., 1999). Consequently, the SpO_2 values recorded during the first 10 s of the transitions from hypoxia to higher P_IO_2 underestimated the actual SaO_2 values. Nevertheless, we should emphasize that the readings of our pulse oximeter were closely correlated to the SaO_2 values, when measured simultaneously under steady conditions in the same subjects included in this study ($SaO_2 = 1.005 \times SpO_2 - 0.76$, $n = 74$, SaO_2 range: 53.9–96.5%, $R^2 = 0.99$). Since during the transitions VO_2 increased linearly with the increase of P_IO_2 (**Figure 6A**), oxygen transport from the lungs to the muscles and the central nervous system must have also been enhanced.

In summary, this investigation demonstrates that close to task failure, MA is lower during IE test to exhaustion in SAH than in normoxia. We have shown that increasing P_IO_2 at exhaustion reduces fatigue and allows for the continuation of exercise in moderate and severe acute hypoxia, regardless of the effects of oxygenation on muscle activation. In hypoxia, MA at task failure is increased within 10 s of oxygenation when task failure occurred at levels of hypoxia equivalent to an altitude close to 5200 m above sea level ($P_IO_2 \sim 73$ mmHg), and when the P_IO_2 is increased to levels ≥ 92 mmHg and SaO_2 above 70%. Overall, these findings indicate that one of the central mechanisms by which severe hypoxia may cause central fatigue and task failure is by reducing the capacity for maximal muscle activation. The fact that exercise could be continued at exhaustion in severe hypoxia with the administration of a placebo-gas mixture demonstrates that this central mechanism has a cognitive component.

AUTHOR CONTRIBUTIONS

Conception and design of the experiments: JC; pre-testing, experimental preparation, data collection, and analysis: RT, DM, JL, IP, JP, and JC; EMG analysis: RT, MG, and MI. The first version of the manuscript was written by RT and JC. All co-authors read and approved the final version of the manuscript.

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High-Intensity Exercise in Hypoxia: Is Increased Reliance on Anaerobic Metabolism Important?

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INTRODUCTION

Hypoxic training strategies to optimize physiological exercise responses have been extensively investigated, although often with limited performance benefits over the equivalent normoxic training (Roels et al., 2007). Recently, novel methods including intermittent hypoxic resistance training (IHRT) and repeat sprint training in hypoxia (RSH) have begun to receive research attention. Early results indicate that IHRT can augment muscle hypertrophy and strength compared to normoxic training (Nishimura et al., 2010; Manimmanakorn et al., 2013a,b), while RSH improves fatigue resistance, resulting in an increased capacity for repeated maximal efforts (Galvin et al., 2013; Faiss et al., 2013b). Although performing these high-intensity activities in hypoxia appears to provide some benefits for training adaptations, the mechanisms underpinning these responses are not fully understood. The beneficial responses to high-intensity exercise in hypoxia may result from a greater reliance on anaerobic metabolism, suggesting that increased metabolic stress may drive (or at least contribute to) these adaptations (Faiss et al., 2013b; Scott et al., 2015a). Considering the likely importance of metabolic stress on adaptation to IHRT and RSH strategies, the purpose of this paper is to briefly discuss the potential benefits of high-intensity training in hypoxia with reference to the role of anaerobic processes.

IMPACTS OF HYPOXIA ON METABOLIC PROCESSES

Both resistance exercise and repeated sprints are characterized by multiple, maximal or near-maximal efforts separated by incomplete recovery periods. Performance during such activities is largely reliant on phosphocreatine (PCr) resynthesis rate (Girard et al., 2011). These high-energy phosphates provide a fuel source during brief high-intensity efforts (Girard et al., 2011), and are therefore necessary to mitigate a decline in performance across repeated efforts. Oxygen availability is an important moderator for PCr resynthesis kinetics, with slower PCr recovery under hypoxic (fraction of inspired oxygen [F_IO_2] = 10%) compared with normoxic conditions, and accelerated PCr recovery rates when breathing hyperoxic air (F_IO_2 = 100%) (Haseler et al., 1999). During IHRT and RSH, participants recover between efforts in hypoxia, likely impairing PCr resynthesis. Therefore, subsequent efforts are performed under progressively more challenging circumstances, with less energy contribution from PCr stores. This also rationalizes why performing a single set of repeat sprint exercise is relatively unaffected by hypoxic conditions (Goods et al., 2014), whereas impaired performance is observed across multiple sets (Balsom et al., 1994; Billaut et al., 2013; Kon et al., 2015; Morrison et al., 2015).

Exacerbated deoxygenation of skeletal muscle tissue in hypoxia has also been shown to increase reliance on glycolytic rather than aerobic energy production (Bowtell et al., 2014). Although it is not clear regarding the actual contributions of different systems to energy production during IHRT

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or RSH, research has demonstrated that short duration running performance can be maintained in hypoxia due to a shift toward anaerobic metabolism (Weyand et al., 1999). Corroborating these findings, research investigating both IHRT (Kon et al., 2010, 2012) and RSH (Goods et al., 2014; Morrison et al., 2015) has observed increased concentrations of blood lactate (a by-product of glycolysis) when exercising in hypoxia compared with normoxia. These data suggest that a decrease in oxygen availability during IHRT and RSH increases the contribution of anaerobic metabolism to total energy production, and may result in performance impairment, as first demonstrated by Balsom et al. (1994) over 20 years ago.

Nevertheless, it should be acknowledged that some research has not observed hypoxia to increase markers of metabolic stress during resistance exercise (Kurobe et al., 2015; Yan et al., 2016) or repeat sprint training (Billaut et al., 2013; Gatterer et al., 2014; Goods et al., 2015). In IHRT research, these contrasting findings may result from differences in the structure of exercise. If sufficient repetition volume during sets is not performed, the contraction time during which metabolites accumulate is decreased, and if inter-set rest periods are too long there is a greater chance for intramuscular metabolites to be removed into circulation and PCr resynthesis to occur (Scott et al., 2014, 2015a). Likewise, if adequate recovery is given between efforts during RSH training protocols, the replenishment of PCr stores may occur despite the hypoxic conditions (Girard et al., 2011). Considering that metabolic stress is a likely moderator of adaptation to hypoxic training, optimal training responses may require that exercise and work:rest ratios are structured to exaggerate anaerobic energy production and limit recovery between repeated sets.

ROLES OF METABOLIC STRESS IN ADAPTATIONS TO EXERCISE

Increased metabolic stress is proposed to stimulate various physiological processes associated with muscle hypertrophy (Schoenfeld, 2013). Nevertheless, the exact mechanisms by which this occurs are not fully understood. One possible explanation is an increase in motor unit recruitment. It is possible that metabolic acidosis causes premature fatigue in the fibers initially recruited during exercise, resulting in the activation of additional motor units to maintain the same level of force generation (Manini and Clark, 2009; Manimmanakorn et al., 2013b). This is supported by recent research that has demonstrated heightened integrated electromyography response during moderate-load IHRT compared to the same exercise in normoxia (Scott et al., 2016). If more motor units are recruited during training, a larger portion of the muscle will be stimulated to adapt (Scott et al., 2015a). Cell swelling is another potential mediator for muscle hypertrophy, resulting from metabolite accumulation within the cells and a resultant inflow of water to equilibrate the osmotic gradient (Loenneke et al., 2012). Cellular swelling can increase protein synthesis and decrease protein degradation in a range of cell types (Lang et al., 1998), and it is possible that similar responses occur in muscle cells (Loenneke et al., 2012). Finally, increased growth hormone concentrations have been reported following IHRT protocols (Kon et al., 2010, 2012, 2014), which may be caused by increased lactate build-up and/or metabolic acidosis (Loenneke et al., 2010). However, the role of exercise-induced endocrine responses may not have anabolic effects in healthy individuals as once thought (West and Phillips, 2010), and further research is required to clarify this and other potential mechanisms for hypertrophy during IHRT.

The additional stress associated with hypoxia during repeat sprint training has been demonstrated to improve glycolytic activity in muscle (Girard et al., 2011). This adaptation may be linked to an increased expression of glycolytic enzymes, such as phosphofructokinase (Puype et al., 2013), lactate dehydrogenase (Faiss et al., 2013b) and enzymes involved in pH regulation, such as carbonic anhydrase (Faiss et al., 2013b). Increased metabolic stress can also trigger adaptations that improve pH regulation and enhance blood buffering capacity following repeat sprint training (Girard et al., 2011; Faiss et al., 2013b). Likewise, the creation of a metabolically stressful environment signals adaptive processes that enhance oxygen utilization (via improved blood perfusion) (Casey and Joyner, 2012; Montero and Lundby, 2015) and delivery within the skeletal muscle to facilitate PCr resynthesis during recovery periods (Haseler et al., 1999; Faiss et al., 2013b). Finally, improved fast twitch fiber recruitment similar to that postulated for IHRT is also thought to play a role in enhanced exercise performance following RSH (Faiss et al., 2013a). It is therefore not surprising that the addition of hypoxia to place a further metabolic strain during repeat sprint training has been investigated with promising results regarding performance outcomes. While further research is needed to fully understand how metabolic stress may improve sea-level repeat sprint ability following RSH, the greater metabolic load imposed by hypoxia suggests that it does play an important part in the development of fatigue resistance within skeletal muscle during intense exercise.

RECOMMENDATIONS AND CONSIDERATIONS

Considering the apparent importance of metabolic stress on adaptations to IHRT and RSH, the actual exercise performed should be structured with this in mind. For IHRT, substantial repetition volume is likely required during sets to provide sufficient time-under-tension during which metabolic stress can accumulate. Researchers have shown that IHRT causes significant decreases in minimal oxygenation levels in working muscles (Kon et al., 2010), which indicates a more hypoxic intramuscular environment. This would theoretically place more emphasis on anaerobic energy production, increasing the concentration of metabolic by-products (Kon et al., 2010, 2012). If brief inter-set rest periods are implemented, it may be possible to attenuate the clearance of these metabolites, meaning that the next set would begin with already elevated metabolite concentrations within the muscles. Furthermore, brief rest periods would also not allow for PCr stores to be resynthesized to the same levels in hypoxia as in normoxia, whereas extended recovery periods

may allow for similar PCr recover, irrespective of hypoxia (Scott et al., 2015a). Current evidence suggests that IHRT should be structured using light to moderate loads (20–70% 1-repetition maximum), which allow for substantial repetitions in each set (10–30 repetitions) and short recovery periods (30–60 s) (Scott et al., 2015a). However, the optimal level of hypoxia to use during IHRT has not yet been determined, and this could obviously have a large impact on training adaptations.

For RSH, an important consideration appears to be the level of hypoxia used, with extreme hypoxic conditions (i.e., $F_IO_2 \leq 13\%$) drastically compromising performance capacity (Goods et al., 2014). From a practical point of view, Gatterer et al. (2014) demonstrated that completing shuttle runs in the confined space of a hypoxic chamber is a viable option to gain the benefits of RSH while maintaining movement specificity. Brocherie et al. (2015) recently used this strategy with success, demonstrating that elite hockey players completing over-ground running RSH in an inflatable hypoxic marquee were able to improve and maintain repeated sprint ability for at least 3 weeks after a RSH intervention. This is critical, as previous researchers have identified limited crossover effects of RSH between cycling and running modalities (Goods et al., 2015). Regarding the actual exercise prescription for RSH, it seems that multiple sets of repeated sprints are required to observe increases in metabolic by-products (Morrison et al., 2015) and longer efforts (>6 s) are likely to have a larger impact on anaerobic metabolism (Faiss et al., 2013b; Puype et al., 2013). Additionally, as proposed for IHRT, short incomplete recoveries via exaggerated work:rest ratios (1:2–1:3) may be more successful for increasing glycolytic stress and therefore performance outcomes (Faiss et al., 2013b) than protocols employing work:rest ratios more commonly used for repeated sprint ability tests (1:5+) (Goods et al., 2015). Nevertheless, it must be acknowledged that some studies have reported varied effects of RSH on metabolic adaptation and performance improvements (Galvin et al., 2013; Brocherie et al., 2015; Goods et al., 2015). Further research is therefore needed to provide conclusive recommendations regarding RSH implementation.

It would also be remiss not to highlight that there are some potential limitations associated with IHRT and RSH strategies. It is possible that the additional stress of hypoxia may result in large performance decrements during training, which could mitigate any hypoxia-mediated benefits from such training. Decreases in concentric velocity during resistance exercise sets has been very strongly related to blood lactate concentration (a marker of anaerobic metabolism; $r = 0.93$–0.97) (Sánchez-Medina and González-Badillo, 2011), and it would therefore be expected that hypoxia-mediated increases in metabolic stress might cause a decline in resistance exercise performance. Nevertheless, our group has not observed hypoxia to impact negatively on performance during high-load resistance exercise (Scott et al., 2015b). Furthermore, several studies have reported an inability to match peak speeds, mean power output or mechanical work performed during RSH compared to normoxic repeat sprint training across an entire session (Billaut et al., 2013; Goods et al., 2014; Morrison et al., 2015). It has also been highlighted that central nervous system fatigue may play a role in decreased performance during hypoxic repeat-sprint exercise, indicating anticipatory central regulation of exercise performance (Billaut et al., 2013). This reduction in performance may attenuate the neuromuscular load of the sprint exercise, and should be considered in the planning and periodization of RSH training. The specific work:rest ratios and accumulated volume to achieve metabolic overload will also be dependent on the prior training status of each individual, which could result in different training adaptations between athletes. Similarly, it is possible that physiological responses to repeat sprint exercise may differ between males and females (Billaut and Bishop, 2009), which could result in altered training adaptations during RSH and should be examined further.

CONCLUSIONS

Adding the physiological stress of hypoxia during exercise essentially makes training more reliant on anaerobic pathways. Recent advances in scientific understanding of IHRT and RSH suggest that this metabolic stress may be an important moderator of adaptations to these novel training strategies, albeit via different mechanisms. It is likely that increased metabolic stress during IHRT causes increased motor unit recruitment and cellular swelling, which may drive (at least partially) hypertrophy of skeletal muscle (Schoenfeld, 2013). During RSH, higher intramuscular concentrations of metabolites may improve glycolytic activity, PCr resynthesis rate, oxygen utilization, and fast twitch muscle fiber behavior, effectively promoting peripheral fatigue resistance (Girard et al., 2011; Casey and Joyner, 2012; Faiss et al., 2013b).

AUTHOR CONTRIBUTIONS

BS, KS, and PG were involved in manuscript conceptualization, writing, and editing.

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Changes in Muscle and Cerebral Deoxygenation and Perfusion during Repeated Sprints in Hypoxia to Exhaustion

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During supramaximal exercise, exacerbated at exhaustion and in hypoxia, the circulatory system is challenged to facilitate oxygen delivery to working tissues through cerebral autoregulation which influences fatigue development and muscle performance. The aim of the study was to evaluate the effects of different levels of normobaric hypoxia on the changes in peripheral and cerebral oxygenation and performance during repeated sprints to exhaustion. Eleven recreationally active participants (six men and five women; 26.7 ± 4.2 years, 68.0 ± 14.0 kg, 172 ± 12 cm, 14.1 ± 4.7% body fat) completed three randomized testing visits in conditions of simulated altitude near sea-level (~380 m, F_IO_2 20.9%), ~2000 m (F_IO_2 16.5 ± 0.4%), and ~3800 m (F_IO_2 13.3 ± 0.4%). Each session began with a 12-min warm-up followed by two 10-s sprints and the repeated cycling sprint (10-s sprint: 20-s recovery) test to exhaustion. Measurements included power output, vastus lateralis, and prefrontal deoxygenation [near-infrared spectroscopy, delta (Δ) corresponds to the difference between maximal and minimal values], oxygen uptake, femoral artery blood flow (Doppler ultrasound), hemodynamic variables (transthoracic impedance), blood lactate concentration, and rating of perceived exertion. Performance (total work, kJ; −27.1 ± 25.8% at 2000 m, $p < 0.01$ and −49.4 ± 19.3% at 3800 m, $p < 0.001$) and pulse oxygen saturation (−7.5 ± 6.0%, $p < 0.05$ and −18.4 ± 5.3%, $p < 0.001$, respectively) decreased with hypoxia, when compared to 400 m. Muscle Δ hemoglobin difference ([Hbdiff]) and Δ tissue saturation index (TSI) were lower ($p < 0.01$) at 3800 m than at 2000 and 400 m, and lower Δ deoxyhemoglobin resulted at 3800 m compared with 2000 m. There were reduced changes in peripheral [Δ[Hbdiff], ΔTSI, Δ total hemoglobin ([tHb])] and greater changes in cerebral (Δ[Hbdiff], Δ[tHb]) oxygenation throughout the test to exhaustion ($p < 0.05$). Changes in cerebral deoxygenation were greater at 3800 m than at 2000 and 400 m ($p < 0.01$). This study confirms that performance in hypoxia is limited by continually decreasing oxygen saturation, even though exercise can be sustained despite maximal peripheral deoxygenation. There may be a cerebral autoregulation of increased perfusion accounting for the decreased arterial oxygen content and allowing for task continuation, as shown by the continued cerebral deoxygenation.

Keywords: repeated sprint ability, altitude, oxygenation, NIRS, maximal exercise, convection, diffusion, blood flow

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INTRODUCTION

During exercise, the circulatory system is challenged to improve oxygen delivery of the working tissues. Oxygen delivery is known to be regulated by physiological mechanisms of both convective (as decrease in arterial oxygen content in hypoxic conditions due to a lower inspired fraction of oxygen; di Prampero, 2003; Sweeting et al., 2017; Villar and Hughson, 2017) and diffusive (as blood flow is regulated via vascular vasodilation based on the metabolic demand of exercise and transports to the muscle for perfusion and to the mitochondria for energy metabolism; Calbet, 2000; di Prampero, 2003; Laughlin and Joyner, 2003; Walker et al., 2007) factors. It has been demonstrated that diminishing the oxygen availability has a detrimental effect on endurance performance as well as on the ability to repeat maximal and short sprints without adequate recovery (Balsom et al., 1994; Smith and Billaut, 2010, 2012; Girard et al., 2011; Billaut and Buchheit, 2013).

As exercise intensity increases, there is a progressive muscle deoxygenation to a minimal point or plateau close to maximal power output or exhaustion (Grassi et al., 1999; Neary et al., 2001; Subudhi et al., 2007). In hypoxic conditions, this plateau in deoxyhemoglobin concentration has been considered an indication of maximal skeletal muscle oxygen extraction as a product of reduced oxygen availability (Esaki et al., 2005; Legrand et al., 2005). The peripheral muscle tissue was shown to regulate this oxygen extraction in order to longer maintain the balance between oxygen delivery and consumption in hypoxic conditions (Subudhi et al., 2007; Smith and Billaut, 2010, 2012). Researchers have also reported that maintaining a high muscle oxygen extraction during low arterial oxygen pressure conditions facilitates the development of hypoxemia in trained individuals (Van Thienen and Hespel, 2016).

Systemic hypoxemia is considered to be a strong contributor to cerebral deoxygenation, (Nielsen et al., 2002; Amann and Calbet, 2008; Amann and Kayser, 2009) which has been shown to occur also during repeated sprint exercise (Billaut and Smith, 2010). Cerebral deoxygenation has been considered as an influential factor for decreasing exercise intensity or cessation of exercise (Smith and Billaut, 2010). Furthermore, low brain oxygenation due to insufficient oxygen delivery and/or lower pressure gradient of arterial oxygen has an influence on the diffusive delivery of oxygen to the sarcomere and mitochondria, which may induce a central fatigue (Amann and Calbet, 2008). The arterial blood pressure (i.e., cerebral perfusion pressure) increases during exercise, and with rapid or forceful muscle contractions may exceed the limits of cerebral autoregulation (maintenance of blood flow to the brain under conditions of changing blood pressure), thus exposing a potential risk of high blood pressure combined with increased blood flow in the brain (Bill and Linder, 1976; MacDougall et al., 1985; Calbet et al., 2015a; Curtelin et al., 2017). In addition, cerebral blood flow provides an important signal to the central nervous system (CNS) which may become a supplemental limiting factor for exercise at altitude in addition to cardiorespiratory capacity and muscle fatigue (Kayser, 2003; Imray et al., 2005). Moreover, the CNS is sensitive to the factors of partial pressure of arterial oxygen, arterial oxygen content, and arterial oxygen saturation, which together (along with many other factors) influence the cardiac output and thus have an impact on brain function (Calbet et al., 2003). Indeed, cerebral function during exercise (especially supramaximal intensity) may have a large influence on fatigue in addition to the decrease in peripheral muscle performance (Shibuya et al., 2004; Amann et al., 2007; Subudhi et al., 2007; Amann and Calbet, 2008; Smith and Billaut, 2010). However, there is still uncertainty regarding cerebral oxygenation and the effect on performance as well as cessation of exercise during repeated sprints in hypoxia.

Changes in oxygenation and the hemodynamics of these tissues can be non-invasively recorded using near-infrared spectroscopy (NIRS) for real-time measures (Van Beekvelt et al., 2001). Previous reports have examined peripheral and cerebral oxygenation with NIRS during repeated sprint exercise, however, none of these studies have been performed with repeated sprint exercise to exhaustion in hypoxic conditions [series of repeated sprints, (Racinais et al., 2007; Smith and Billaut, 2010, 2012; Billaut and Buchheit, 2013); or incremental ramp test to exhaustion, (Amann et al., 2007; Subudhi et al., 2007)]. In addition, recent research has eluded that there may be greater blood volume shifts in the muscle during repeated sprint exercise possibly due to greater arteriolar dilation together with increased capillary volume (De Smet et al., 2017). Therefore, there are factors contributing to the end of exercise within maximal repeated sprints to exhaustion which remain unknown.

Thus, the aim of the present study was to investigate changes in peripheral and cerebral oxygenation during maximal repeated sprints to exhaustion as well as the physiological effects of different levels of hypoxia. The hypothesis was that performance would be impaired in hypoxia due to decreased convective oxygen delivery. Additionally, the changes in peripheral and cerebral de/re-oxygenation during sprints/recoveries were hypothesized to be greater as hypoxia increased, and that cerebral oxygenation variables may challenge blood flow regulation at the end of exercise.

Abbreviations: F_IO_2, fraction of inspired oxygen; NIRS, near-infrared spectroscopy; Δ, delta change over time; RSAT, repeated sprint ability test; $\dot{V}O_2$, maximal oxygen uptake; $\dot{V}_E$, minute ventilation; $\dot{V}_E/\dot{V}O_2$, ventilatory equivalent for oxygen; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for carbon dioxide; RER, respiratory exchange ratio; BF, breathing frequency; SV, stroke volume; HR, heart rate; Q, cardiac output; SVR, systemic vascular resistance; EDV, end diastolic volume; EF, ejection fraction; RPE, rate of perceived exertion; O_2Hb, oxyhemoglobin; HHb, deoxyhemoglobin; tHb, total hemoglobin; Hbdiff, hemoglobin difference; TSI, tissue saturation index; $P_{a,CO2}$, partial pressure of arterial carbon dioxide; $P_{a,O2}$, partial pressure of arterial oxygen.

METHODS

Participants

Eleven healthy, recreationally active volunteers participated in this study (six men and five women; 26.7 ± 4.2 years, 68.0 ± 14.0 kg, 172 ± 12 cm, 14.1 ± 4.7% body fat). Participants were required to train at least 4 h per week and be accustomed to maximal intensity exercise. Exclusion criteria for participation included any skeletal or muscular injury in the last 3 months,

pain, or any other medical condition which could compromise the study. Participants gave written informed consent after being informed of the procedures and risks involved. The experimental protocol was approved by the Ethical Commission for Human Research CER-VD 138/15 and conducted according to the Declaration of Helsinki.

Study Design

In a randomized, single-blinded experimental protocol, participants reported to the laboratory for a total of four sessions (one familiarization and three testing visits). The testing visits were performed in simulated altitude of sea level (∼380 m, F_IO_2 20.9%), ∼2000 m (F_IO_2 16.5 ± 0.4%), and ∼3800 m (F_IO_2 13.3 ± 0.4%). Each session was completed in a normobaric hypoxic chamber (ATS Altitude Training, Sydney, Australia) built in the laboratory. This chamber utilizes a system containing a compressor connected to air filters which enable the regulation of the level of oxygen (simulated altitude settings as noted above). Participants were asked to avoid strenuous activity as well as caffeine or alcohol consumption 24 h before each visit. All visits were scheduled at the same time of day and at least 48 h apart to limit fatigue.

Familiarization

During the familiarization visit, anthropometric data (body height, body mass, and skin fold measurement) were collected along with the completion of the informed consent and health questionnaires. Skin fold measurements were obtained by an experienced technician using the seven-site formula from the 2014 ACSM guidelines (ACSM, 2014). Afterwards, participants were seated on an electronically braked cycling ergometer (Lode Excalibur Sport Ergometer, Lode B.V., Netherlands) and dimensions were recorded for standardization during subsequent sessions. After a 5-min warm-up at 1.5 $W \cdot kg^{-1}$, participants performed two 10-s maximal sprints with 3 min of active recovery between. Following an additional 5-min passive recovery, participants were familiarized with the repeated sprint ability test (RSAT), as described in detail below.

Testing Visits

The protocol of the testing visit is illustrated in **Figure 1**. Each session began in the normobaric hypoxic chamber with a 12-min warm-up (6 min at 50 W, 6 min at 100 W) at a cadence of 85 rpm. Following which, the two maximal 10-s warm-up sprints were performed (similar to the familiarization visit) with 3 min of active recovery between sprints. After a 5-min passive recovery, the measurements for pre-RSAT were collected. These measurements (described in detail below) included resting Doppler blood flow and estimates of cardiovascular function synchronized at the same moment. Participants were then fitted with a mask to measure oxygen uptake. Participants then performed the RSAT to exhaustion. Upon completion of the test, measurements were taken in the following sequence: Doppler blood flow at 1-min from end of RSAT in synchronization with cardiovascular function, blood lactate concentration, and rating of perceived exertion (RPE) values.

Repeated Sprint Ability Test

After the normalization and warm-up period described above, participants performed the RSAT as previously described previously (Faiss et al., 2013). Participants began pedaling at 20 W with a cadence of 85 rpm for 1 min, followed immediately by the RSAT of 10-s all-out maximal sprint and 20-s active recovery (1:2 work-to-rest ratio) until volitional exhaustion or task failure (cadence <70 rpm) similar to Faiss et al. (2013). Immediately at the end of each 10-s sprint, the ergometer automatically switched to a resistance of 20 W for the 20-s recovery. Participants were given very strong verbal encouragement and were not given any indication of the number of sprints performed. All sprints were performed using the "Wingate mode" from the manufacturer with an individually fixed torque factor of 0.8 $Nm \cdot kg^{-1}$. Participants were instructed to perform each sprint as hard and fast as possible and perform as many sprints as possible while also maintaining a similar body position. In order to avoid any pacing strategy, the first two sprints were controlled to obtain at least 95% of the best sprint from the two warm-up sprints performed, which was the case for each test. Mean power (W), number of sprints performed, and total work (kJ) were obtained for further analysis, as well as the calculation of fatigue index or percent decrement ($S_{dec}(\%) = [1 - ((S_1 + S_2 + S_3 + \ldots + S_{final})/(S_{best} \times \text{number of sprints}))] \times 100$), where S_1 corresponds to sprint 1, etc., (Glaister et al., 2008). During RSAT, pulse oxygen saturation was measured at the earlobe with an oximeter (8000Q2 Sensor, Nonin Medical Inc., Amsterdam, The Netherlands) and recorded at 5 Hz, the minimum of which was used for further analysis. The RPE was evaluated using the Borg scale (6–20) as a perception of effort in both the legs and the breathing immediately after the test.

Metabolic Measurements

Breath-by-breath pulmonary gas-exchange data were collected continuously (Medgraphics CPX, Loma Linda, CA, USA). Oxygen consumption ($\dot{V}O_2$), ventilation ($\dot{V}_E$), ventilatory equivalent for oxygen ($\dot{V}_E/\dot{V}O_2$), and for carbon dioxide ($\dot{V}_E/\dot{V}CO_2$), respiratory exchange ratio (RER), and breath frequency (BF) were computed and stored for further analyses. The system was calibrated with a 3-L syringe (M9474, Medikro Oy, Finland) and known gas mixtures of O_2 and CO_2 prior to each measurement. Heart rate was monitored at 5 Hz with a telemetry based heart rate monitor (Polar RS400, Kempele, Finland) and the maximum recorded was used for further analysis. During the RSAT, the highest 30-s average of oxygen uptake was computed and used for further analysis. In addition, blood lactate concentration was assessed at the earlobe post-RSAT. After the skin was cleaned and dried, a lancet was used to take a small droplet (0.2 µl) into the strip for analysis (Lactate Scout, EKF Diagnostics, GmbH, Leipzig, Germany).

Cardiovascular Measurements

Estimates of cardiovascular variables were obtained throughout the entire protocol using Physioflow® (Manatec type PF05L1, Paris, France). The measuring device is based on a bioimpedance method which continuously calculates stroke volume (SV), heart rate (HR), cardiac output (Q), systemic vascular resistance (SVR),

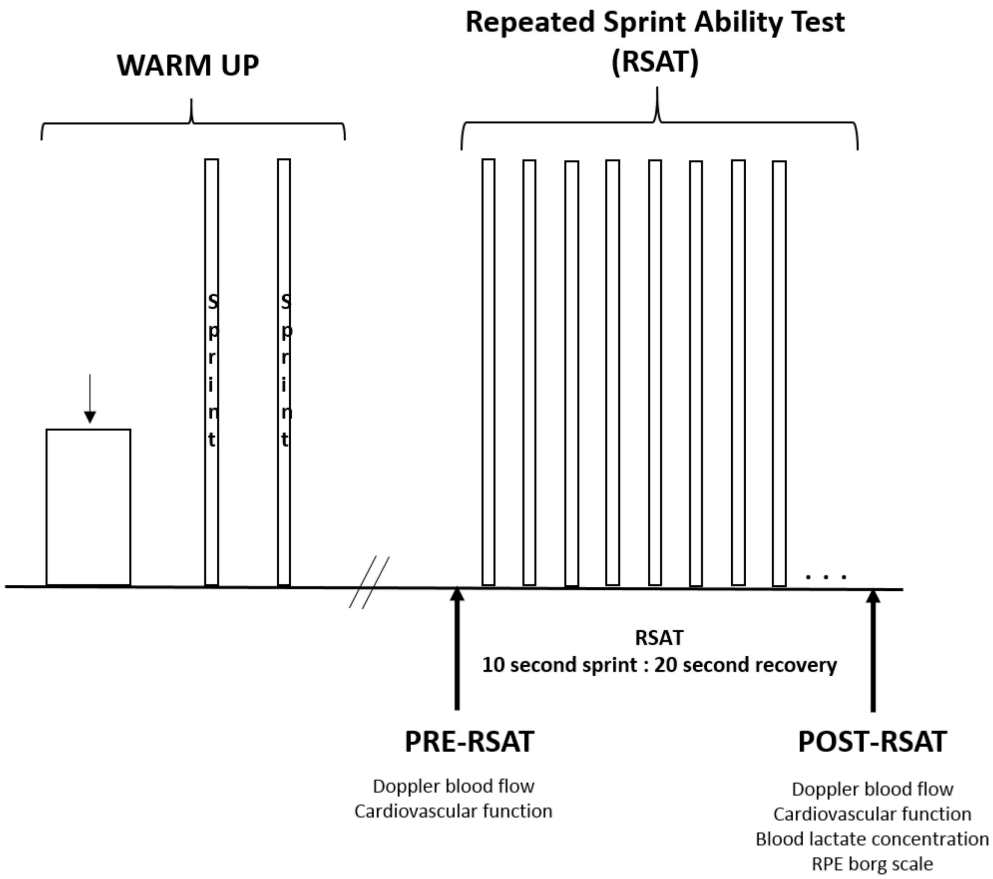

FIGURE 1 | General protocol of warm up and repeated sprint ability test to exhaustion.

end diastolic volume (EDV), and ejection fraction (EF) to detect changes in transthoracic impedance during cardiac ejection. These parameters were analyzed at the same moment (average of 30 s) as the pre- and post- Doppler blood flow measurements. While in the cycling position at rest, Doppler blood flow measurements were collected by an experienced technician on the left femoral artery with a linear probe (L12-5L60N) using EchoWave II 3.4.4 software (Telemed Medical Systems, Telemed Ltd. Lithuania, Milano, Italy) ∼5 min pre-, as well as at 1-min post-RSAT. A video image was obtained for 30 s and subsequent analysis was performed to take an average of 10 frames, meaning a measurement every ∼1.5 s. Measurements of the blood flow were calculated within the software via a measurement of the vessel diameter (mm) and the blood velocity ($cm \cdot s^{-1}$).

Near-Infrared Spectroscopy Measurements

Muscle oxygenation was evaluated using the near-infrared spectroscopy (NIRS) technique as described previously by Boushel and Piantadosi (2000). The PortaMon and PortaLite devices (Artinis, Zetten, The Netherlands) were used to measure muscle oxygenation of the vastus lateralis (PortaMon) and of the prefrontal cortex (PortaLite) at wavelengths between 760 and 850 nm. All devices were placed into a tight transparent plastic wrap to avoid humidity and create a waterproof barrier for proper function and signal quality. The PortaMon was placed on the lower third of the vastus lateralis and attached with double sided tape, then wrapped with tension against the leg to reduce movement during exercise. The position was marked with a permanent pen and images were taken to reproduce the placement in subsequent visits. The PortaLite was attached on the surface of the left prefrontal cortex with double sided tape, then the subject was fitted with a head wrap to create a dark environment and maintain a stable position of the probe. Measurements included a standard differential pathlength factor of 4.0 for the vastus lateralis as there is a lack of any clear standard value for the quadriceps during cycling sprints (Faiss et al., 2013) and 6.0 for the prefrontal cortex, similar to van der Zee et al. (1992) and Amann et al. (2007). All signals were recorded at the maximum frequency for each device (10 Hz for PortaMon and 50 Hz for PortaLite) and then exported at 10 Hz for further analysis (Oxysoft 3.0.53, Artinis, The Netherlands). For analysis, a 4th-order low-pass zero-phase Butterworth filter (cutoff frequency 0.2 Hz) was implemented to reduce artifacts and smooth perturbations in the signal from pedal strokes. Detection of maximum and minimum was performed automatically using

deoxyhemoglobin as reference value for the start point of sprints. This allowed determination of successive sprint and recovery phases to be identified, and sprint phases to be further analyzed. The change (Δ) for each sprint was defined as the difference between maximum and minimum values for each sprint. Delta concentrations of oxyhemoglobin (Δ[O_2Hb]), deoxyhemoglobin (Δ[HHb]), hemoglobin difference (Δ[Hbdiff]), total hemoglobin (Δ[tHb]), and tissue saturation index (ΔTSI, %) were obtained. Finally, the analysis was normalized to the duration of the set to exhaustion; i.e., percentage of sprints performed (i.e., 20, 40, 60, 80, 100%), and a linear interpolation was calculated when there was a fractional number of sprints, since each participant performed a different number of sprints in each condition.

Statistical Analysis

Cardiovascular estimates and blood flow measurements were evaluated with a linear mixed effects analysis of the relationship between condition (400, 2000, 3800 m) and time (pre or post). Fixed effects included condition and time, while participant was set as a random effect. Measurements of oxygenation were also evaluated with a linear mixed model analysis with fixed effects of condition and set duration (20, 40, 60, 80, 100% of sprints performed) with participants as the random effect. The remaining variables including: performance, metabolic gas exchange, pulse oxygen saturation, blood lactate, and RPE were also analyzed with a linear mixed model setting condition as the fixed effect and participant as the random effect. Visual inspection of residual plots did not reveal obvious deviations from homoscedasticity or normality. All analyses were performed using R (R Core Team, 2017, Foundation for Statistical Computing, Vienna, Austria) and nlme4 (Pinheiro et al., 2017). *P*-values were set to 0.05 and were obtained by likelihood ratio tests of the full model with the effect in question against the model without the effect in question. Contrasts were obtained using least-squares means for mixed models [library lsmeans, (Lenth, 2016)] employing Tukey method. Values are represented in tables and figures as mean ± standard deviation.

TABLE 1 | Performance and respiratory values during repeated sprint test to exhaustion in simulated altitude of 400, 2000, and 3800 m.

	400 m	2000 m	3800 m
Number of sprints	29.8 ± 13.7 34 (13–47)	19.8 ± 10.2$^{\#}$ 38 (9–47)	15.4 ± 9.5$^{\#\#\#}$ 26 (7–33)
Mean power (W)	543 ± 135 403 (332–736)	557 ± 150 456 (344–801)	511 ± 139$^{\&}$ 480 (320–800)
Fatigue index (% decrement)	26.5 ± 7.2 22 (14–36)	23.6 ± 7.1 21 (13–34)	26.4 ± 8.0 28 (12–40)
Total work (kJ)	162 ± 81 256 (50–306)	107 ± 41$^{\#\#}$ 137 (31–168)	78 ± 48$^{\#\#\#}$ 25 (166–191)
Maximal heart rate (bpm)	185 ± 9 29 (174–203)	183 ± 11 34 (165–199)	178 ± 8$^{\#\#\#\&}$ 25 (166–191)
SpO_2 (%)	93.8 ± 4.5 14 (86–100)	86.7 ± 6.4$^{\#}$ 18 (78–96)	76.5 ± 6.0$^{\#\#\#\&\&\&}$ 18 (70–88)
$\dot{V}O_2$ ($ml{\cdot}kg^{-1}{\cdot}min^{-1}$)	40.1 ± 4.1 14.0 (34.5–8.5)	36.4 ± 4.3$^{\#\#\#}$ 11.5 (30.3–41.8)	32.0 ± 3.7$^{\#\#\#\&\&\&}$ 9.8 (27.0–36.8)
RER	1.11 ± 0.06 0.19 (1.01–1.20)	1.19 ± 0.08$^{\#\#}$ 0.22 (1.10–1.32)	1.22 ± 0.09$^{\#\#\#}$ 0.26 (1.06–1.32)
$\dot{V}_E$ ($L{\cdot}min^{-1}$)	137 ± 28 98.8 (93.6–192.4)	137 ± 30 108.2 (90.6–198.8)	135 ± 35$^{\#\&}$ 117.2 (80.8–198)
$\dot{V}_E/\dot{V}O_2$	50.7 ± 4.5 13.1 (43.1–56.2)	56.0 ± 4.8$^{\#\#\#}$ 14.4 (48.3–62.7)	60.0 ± 6.6$^{\#\#\#\&\&}$ 21.9 (48.4–70.3)
$\dot{V}_E/\dot{V}CO_2$	45.8 ± 4.4 12.4 (39.9–52.3)	47.0 ± 4.1 11.9 (40.4–52.3)	49.3 ± 3.2$^{\#}$ 8.8 (45.0–53.8)
BF ($br{\cdot}min^{-1}$)	66.9 ± 5.0 16 (59–75)	64.2 ± 3.7 13 (58–71)	63.9 ± 6.5 22 (54–76)
RPE legs (Borg 6-20)	17.7 ± 2.1 5 (15–20)	18.5 ± 1.3 4 (16–20)	17.8 ± 1.9 6 (14–20)
RPE breathing (Borg 6-20)	18.3 ± 1.4 4 (16–20)	19.2 ± 0.8 2 (18–20)	18.3 ± 2.3 8 (12–20)
Blood lactate ($mmol{\cdot}L^{-1}$)	9.5 ± 5.2 16.9 (4.6–21.5)	11.6 ± 4.8 14.9 (6.2–21.1)	10.7 ± 5.2 13.7 (4.6–18.3)

Mean ± SD. Range (minimum-maximum). $^{\#\#\#}p < 0.001$, $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ *for difference with 400 m.* $^{\&\&\&}p < 0.001$, $^{\&\&}p < 0.01$, $^{\&}p < 0.05$ *for difference with 2000 m.* SpO_2*, pulse oxygen saturation;* $\dot{V}O_2$*, oxygen uptake; RER, respiratory exchange ratio;* $\dot{V}_E$*, minute ventilation; BF, breathing frequency; RPE, rating of perceived exertion.*

RESULTS

Performance

All performance data are presented in **Table 1**. The number of sprints performed to exhaustion decreased by 26.9 ± 29.5% at 2000 m ($p < 0.05$) and 44.9 ± 22.8% at 3800 m ($p < 0.001$) when compared to 400 m. Similarly, the total work was decreased by 27.1 ± 25.8% ($p < 0.01$) and 49.4 ± 19.3% ($p < 0.001$), respectively. As expected, the pulse oxygen saturation also decreased 7.5 ± 6.0% at 2000 m ($p < 0.05$) and 18.4 ± 5.3% at 3800 m ($p < 0.001$), when compared to 400 m.

Metabolic Responses

Metabolic data are also presented in **Table 1**. During the repeated sprint to exhaustion, the peak oxygen uptake was reduced by 9.3 ± 5.5% at 2000 m ($p < 0.001$) and 19.8 ± 5.7% at 3800 m ($p < 0.001$), when compared to 400 m. There was a higher respiratory exchange ratio (RER, $\dot{V}CO_2/\dot{V}O_2$) with increased altitude ($p < 0.01$). In addition, the ventilatory equivalent for oxygen ($\dot{V}_E/\dot{V}O_2$) increased by 10.6 ± 4.6% at 2000 m and 17.9 ± 5.0% at 3800 m, ($p < 0.001$), respectively.

Cardiovascular Responses

There was a main effect of time (pre-post) for stroke volume (SV, $p < 0.05$), heart rate (HR, $p < 0.001$), and cardiac output (Q, $p < 0.001$) which all increased, while the systemic vascular resistance (SVR) was decreased ($p < 0.001$). There were no main effects of condition and no interactions present. All cardiovascular results are shown in **Table 2**. The average increase in femoral artery blood flow from pre- to post- repeated sprint to exhaustion was 71% at 400 m, 34% at 2000 m, and 24% at 3800 m when compared with the resting measurement pre-RSAT.

Peripheral Oxygenation

As indicated in **Figure 2**, for the vastus lateralis, there was a main effect of condition which resulted in a lower Δ[Hbdiff] and ΔTSI with the highest deoxygenation at 3800 m when compared

TABLE 2 | Average cardiovascular values pre- and 1-min post- repeated sprint ability test (RSAT) representing a main effect difference post-RSAT in simulated altitude of 400, 2000, and 3800 m.

	400 m		2000 m		3800 m	
	Pre-	**Post-**	**Pre-**	**Post-**	**Pre-**	**Post-**
SV	89 ± 11	101 ± 22*	77 ± 16	92 ± 22*	94 ± 18	98 ± 21*
	29 (74–103)	63 (73–136)	52 (52–104)	61 (69–130)	62 (74–136)	63 (76–139)
HR (bpm)	94 ± 17	136 ± 16***	97 ± 16	130 ± 25***	95 ± 16	123 ± 15***
	49 (72–121)	62 (108–170)	55 (69–124)	71 (107–178)	58 (67–125)	44 (101–145)
Q ($L \cdot min^{-1}$)	8.4 ± 2.0	14.0 ± 4.2***	7.6 ± 2.7	12.4 ± 5.5***	8.8 ± 1.9	12.0 ± 2.4***
	6.8 (5.4–12.2)	14.4 (7.9–22.3)	8.1 (4.8–12.9)	14.8 (8.4–23.2)	6.4 (6.3–12.7)	6.6 (8.9–15.5)
SVR ($dyn.s \cdot cm^{-5}$)	817 ± 173	508 ± 140***	999 ± 302	621 ± 182***	843 ± 165	611 ± 101***
	466 (588–1,054)	403 (324–727)	928 (582–1,510)	541 (338–879)	577 (623–1,200)	260 (508–768)
EDV (ml)	114 ± 18	134 ± 31*	107 ± 11	122 ± 22*	124 ± 32	121 ± 25*
	51 (89–140)	90 (91–181)	30 (94–124)	62 (98–160)	99 (95–194)	77 (96–173)
EF (%)	78.7 ± 3.3	77.6 ± 14.2	71.6 ± 12.7	75.6 ± 2.9	76.9 ± 8.3	81.0 ± 3.7
	8.8 (73.6–82.4)	50.8 (40.8–91.6)	37.5 (46.4–83.9)	26.7 (58.9–85.6)	26.3 (64.7–91.0)	11.1 (74.1–85.2)
Blood Flow ($ml \cdot min^{-1}$)[£]	405 ± 276	694 ± 716	633 ± 555	847 ± 588	631 ± 699	782 ± 744
	771 (14–785)	1,990 (11–2,001)	1,836 (9–1,845)	1,626 (138–1,764)	2,013 (5–2,018)	1,913 (19–1,932)

*Mean ± SD Range (minimum-maximum). Measures were obtained at rest prior to RSAT (pre-), and at 1-min post-RSAT. [£]Blood flow for one subject was obtained from the popliteal artery, thus the range of values is high. ***$p < 0.001$, *$p < 0.05$ for difference with pre-. SV, stroke volume; HR, heart rate; Q, cardiac output; SVR, systemic vascular resistance; EDV, end diastolic volume; EF, ejection fraction.*

with 400 and 2000 m ($p < 0.05$). Additionally, the main effect of condition was indicated for Δ[HHb] and Δ[tHb] in which resulted in greater changes in the 2000 m condition ($p < 0.05$) compared with 400 and 3800 m. There was a main effect of set duration near the end of the sprint test to exhaustion ($p < 0.05$) with Δ[Hbdiff], ΔTSI, and Δ[tHb] decreasing toward end of the test. Absolute maximal TSI values are shown in **Figure 4**, with a main effect of hypoxic condition and a continual decrease as hypoxia severity increased ($p < 0.001$) as well as a main effect of set duration near exhaustion ($p < 0.05$). No interactions were present.

Cerebral Oxygenation

In the prefrontal cortex (**Figure 3**), there was a main effect of condition for Δ[HHb] with a smaller decrease (smaller change) at 3800 m when compared with 400 and 2000 m ($p < 0.01$). Additionally, a main effect of condition resulted with greater Δ[Hbdiff] at both 2000 and 3800 m, compared with 400 m ($p < 0.05$). Similarly, there was higher ΔTSI at both 2000 and 3800 m when compared with 400 m ($p < 0.01$). There was a main effect of set duration near the end of the sprint test to exhaustion ($p < 0.05$) with Δ[Hbdiff] and Δ[tHb] increasing in the last 20% of the sprint test. In addition, for Δ[Hbdiff], there was a significant interaction effect between condition and set duration ($F = 2.706$, $p < 0.01$).

DISCUSSION

The main findings of this study were:

1. There was a continual decrease in convective factors of oxygen delivery (e.g., decreases in pulse oxygen saturation and peak oxygen uptake) with increased hypoxia severity, which was linked with impairment in performance (number of sprints and total work) across conditions.
2. There were reduced changes in peripheral oxygenation values at 3800 m indicating a possible limitation of the oxygen transport system (i.e., circulatory) which was non-linear as hypoxia increased, likely indicating different responses as altitude increases. This may suggest a threshold between 2000 and 3800 m regarding the means of oxygen transport, which supports previous research stating 2000 m moderate and 3800 m is high altitude (Bartsch et al., 2008).
3. Cerebral deoxygenation demonstrated greater changes at 3800 m compared with 400 and 2000 m, as well as an increased change in blood volume in the final 20% of the set duration (at exhaustion). This may indicate that central autoregulation occurs in order to continue exercise despite limited peripheral and cerebral oxygen delivery, until a certain point of limited diffusion at which protective mechanisms cease exercise.

Decreased Convective Oxygen Delivery

With increased altitude, there was an expected and logical decrease in convective factors of oxygen transport. Specifically, there was a decreased pulse oxygen saturation (level of the capillary) and decreased peak oxygen uptake (pulmonary and systemic circulation) with conditions of decreased oxygen. Maximal heart rate was also decreased significantly at 3800 m. Interestingly, there was no increase with altitude in minute ventilation or breathing frequency. However, since the exercise was maximal and to exhaustion, any difference between altitude levels would have been minor. The ventilatory efficiency index

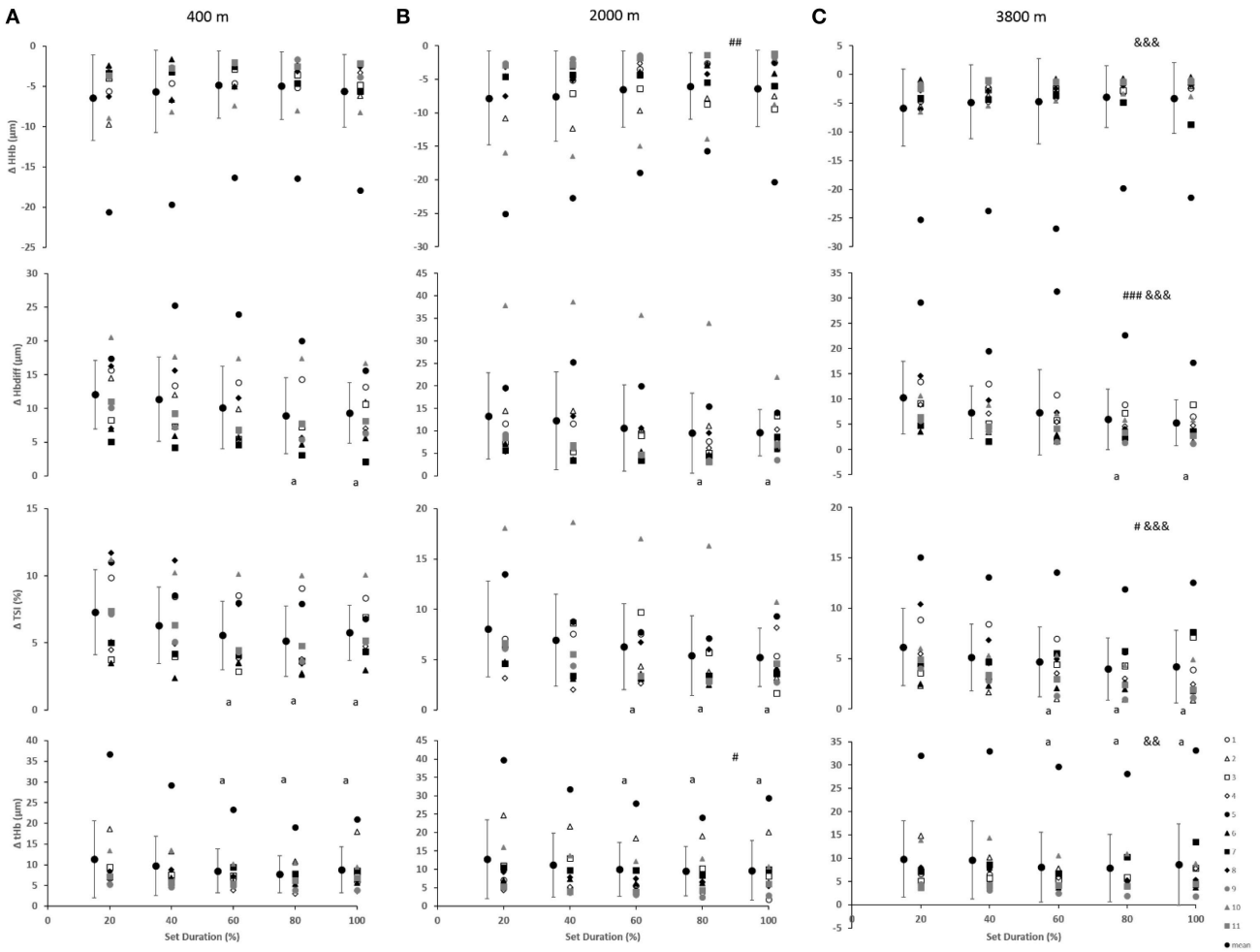

FIGURE 2 | Near-infrared spectroscopy (NIRS) results representing the average maximum-minimum delta (Δ) value during the percentage of sprints completed to exhaustion for the individual response of the vastus lateralis in simulated altitude of **(A)** 400 m, **(B)** 2000 m, and **(C)** 3800 m. Mean ± SD. $^{\#\#\#}p < 0.001$, $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ for difference with 400 m; $^{\&\&\&}p < 0.001$, $^{\&\&}p < 0.01$ for difference with 2000 m. Symbol: a for difference with 20%.

($\dot{V}_E/\dot{V}O_2$) was increased with altitude naturally due to a decrease in oxygen uptake (**Table 1**), similar as previously shown (Calbet et al., 2009). It has been suggested that the first limitation of performance in hypoxia is the delivery of oxygen primarily by factors of convection (which resulted in the present study) and subsequently by means of diffusion capacities at the muscle and mitochondrial (Calbet et al., 2009, 2015b) as well as pulmonary level (Sarkar et al., 2017). Additionally, there were no differences in the hemodynamic response with repeated sprints to exhaustion between altitude conditions (SV, Q, HR, SVR, EDV, EF) and also no acute peripheral vasodilation as noted by femoral arterial blood flow. Peak cardiac output has been shown to remain similar between different levels of hypoxia as a possible counteraction for the reduction in arterial content of oxygen (Calbet et al., 2009). Furthermore, this study demonstrated that the limitation of oxygen delivery resulted in an impairment in performance across conditions. There were no changes in the rating of perceived exertion of the leg or breathing or changes in blood lactate concentration between conditions, indicating that the maximal efforts of the tests were similar despite the differences in total work. These results suggest there are some factor(s) allowing performance to be at least partly maintained despite continued decreases in pulse oxygen saturation and oxygen uptake due to regulatory mechanisms facilitating the oxygen delivery as the severity of hypoxia increases. This can be partly explained by the oxygen-hemoglobin dissociation curve and the shift of the curve based on the affinity for oxygen. As demonstrated by Calbet and colleagues, the offloading of oxygen from hemoglobin does not require a right-shift in the oxygen-hemoglobin dissociation curve indicating less of a role of the Bohr effect (Calbet et al., 2015b).

Limitations of Peripheral Oxygenation

The change in vastus lateralis oxygenation values were less at 3800 m indicating less of a change from maximum to minimum during sprints, meaning there was a further limitation of the oxygen transport system (i.e., circulatory) in comparison to the other conditions of 400 and 2000 m (**Figure 2**). Indeed, the

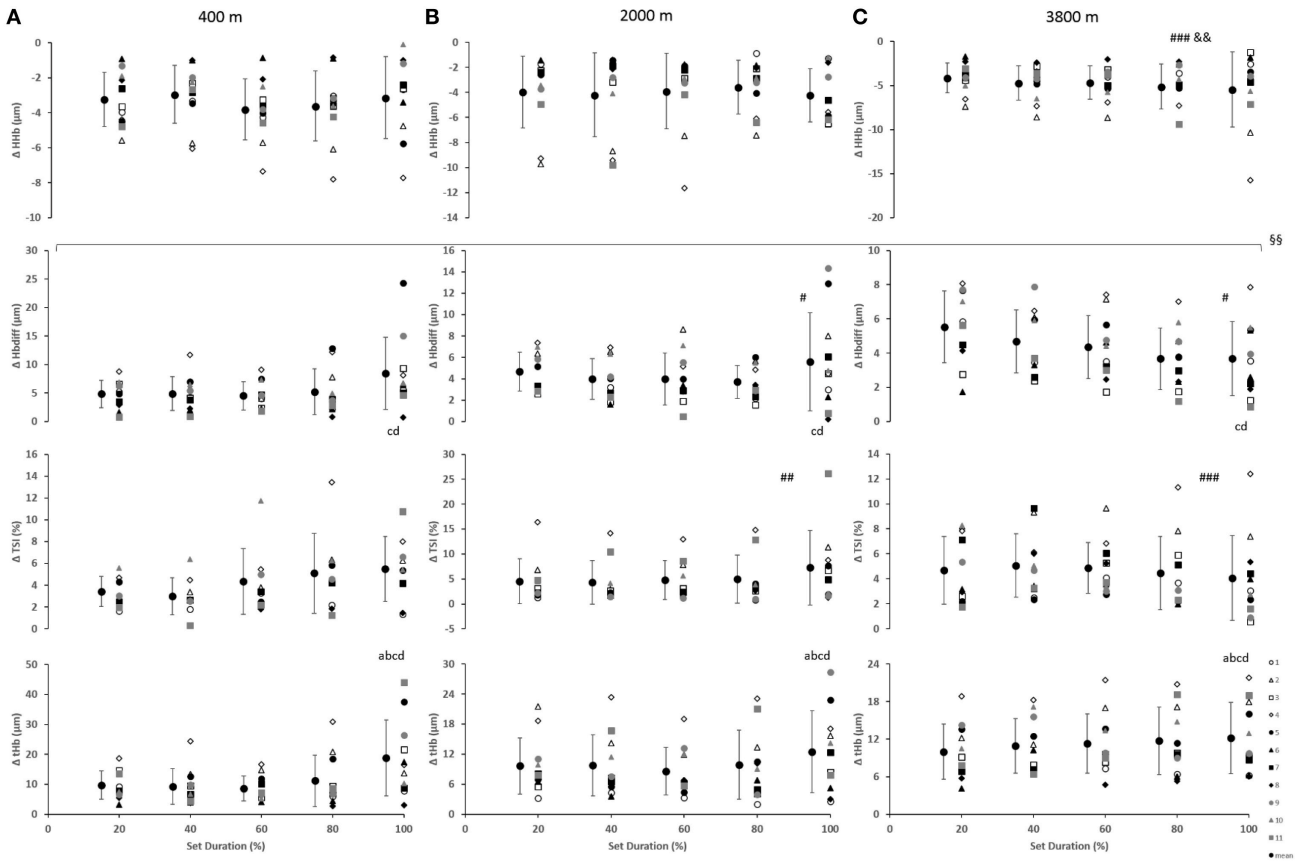

FIGURE 3 | Near-infrared spectroscopy (NIRS) results representing the average maximum-minimum delta (Δ) value during the percentage of sprints completed to exhaustion for the individual response of the prefrontal cortex in simulated altitude of **(A)** 400 m, **(B)** 2000 m, and **(C)** 3800 m. Mean ± SD. $^{\#\#\#}p < 0.001$, $^{\#}p < 0.05$ for difference with 400 m; $^{\&\&}p < 0.01$ for difference with 2000 m. Symbol: a for difference with 20%, b for difference with 40%, c for difference with 60%, and d for difference with 80%. §§Significant ($p < 0.01$) interaction (condition × set duration) was present.

absolute maximal values of TSI indicated a continual limitation of oxygen as hypoxia increased, which decreased toward the end of the test (**Figure 4**). In fact, an inconsistent decrease in Δ[HHb] was demonstrated as the severity of hypoxia increased, indicating a possible threshold between 2000 and 3800 m where peripheral oxygenation becomes limited. An increase in [HHb] in hypoxia has been shown to increase the metabolic demand of exercise (Costes et al., 1996) and is considered as a counteraction for the reduced oxygen availability (Legrand et al., 2005). This is likely due to increased local acidosis reducing hemoglobin's affinity for oxygen through the Bohr effect (Nielsen et al., 2002). Furthermore, previous research has demonstrated that the role of this acidosis is minimal regarding the mechanisms of oxygen offloading during exercise, which supports the result of the current study with no change in blood lactate concentration between conditions, as seen in **Table 1** (Calbet et al., 2015b).

All changes in peripheral oxygenation (Δ[HHb], Δ[tHb], and ΔTSI) were continually decreasing toward the end of the test (**Figure 1**). In the present study, there was a plateau in Δ[HHb], which has been previously suggested to be an indication of maximal skeletal muscle oxygen extraction (Esaki et al., 2005). However, it has also been shown that the peripheral muscle can continue to extract oxygen even in conditions of reduced oxygen availability (Smith and Billaut, 2010, 2012). This supports previous research that suggested the plateau in peripheral deoxygenation was not a reason for the exhaustion and the end of exercise, but rather an indication of an equilibrium between the oxygen delivery and extraction/consumption over higher work rates (Subudhi et al., 2007). This confirms the result of the current study that performance can continue despite limited convective oxygen transport (decreased oxygen uptake and pulse oxygen saturation) as well as despite maximal levels of peripheral deoxygenation. The decreased Δ[HHb] in the vastus lateralis at 3800 m in the present study suggests that a compensatory larger extraction may at least partly counterbalance the lower convective oxygen supply and therefore reduce the diffusion limitation, as previously suggested (Cerretelli, 1976; Wagner, 2000).

Moreover, Amann and colleagues have suggested that when substantial peripheral muscle fatigue has occurred in normoxia or moderate hypoxia, there is no capacity to reverse the magnitude and thus, exercise is terminated via reduction in the central motor output for prevention of further development of peripheral fatigue beyond a critical level (in accordance with

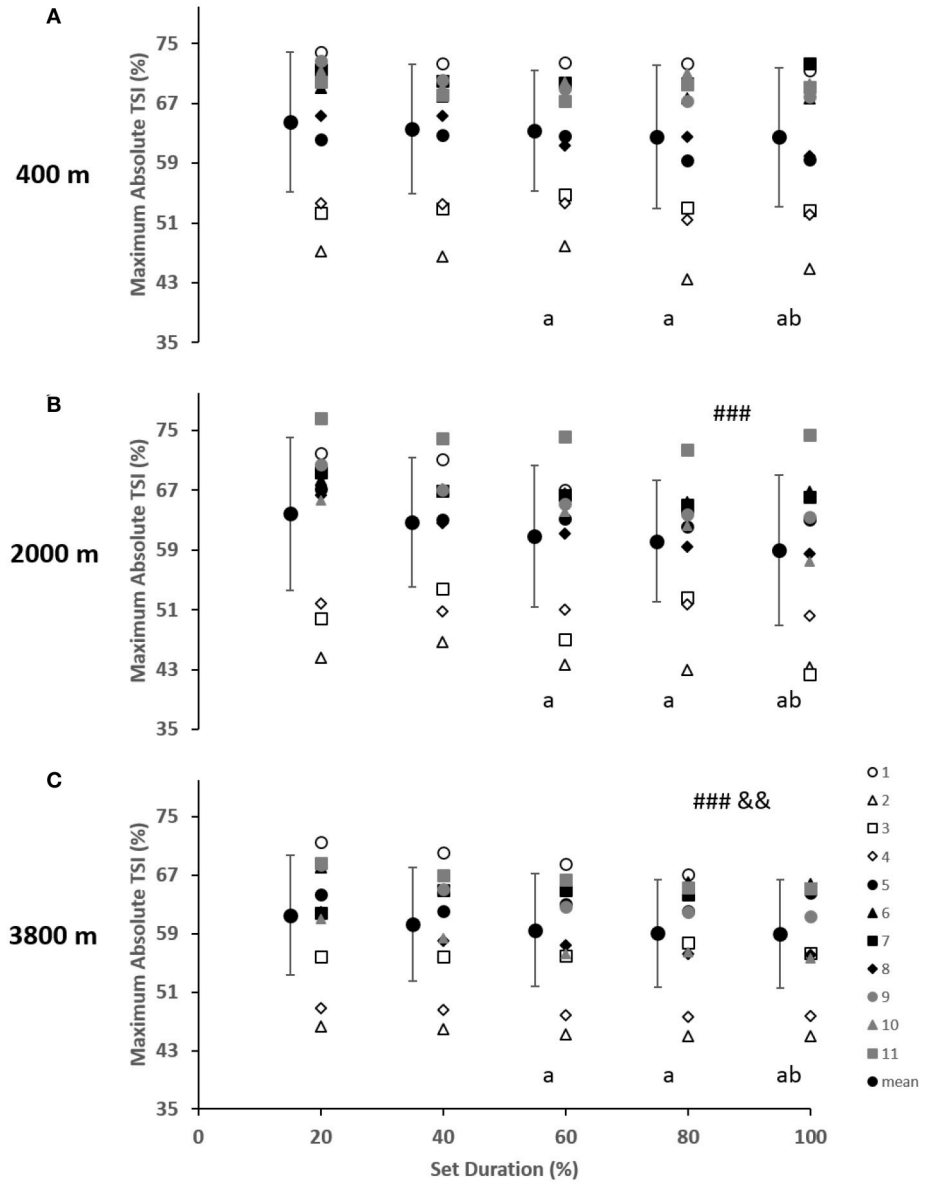

FIGURE 4 | Near-infrared spectroscopy (NIRS) results representing the average maximum value of tissue saturation index (%) during the percentage of sprints completed to exhaustion for the individual response of the vastus lateralis in simulated altitude of **(A)** 400 m, **(B)** 2000 m, and **(C)** 3800 m. Mean ± SD. $^{###}p < 0.001$ for difference with 400 m; $^{\&\&}p < 0.01$ for difference with 2000 m. Symbol: a for difference with 20%, and b for difference with 40%.

their previous research; Amann et al., 2006, 2007; Romer et al., 2007). In addition, researchers have suggested an influence of the central nervous system over the active muscles in hypoxia (Amann et al., 2007), as there is decreased central command at low oxygen fraction (Millet et al., 2009). This mechanism could partially contribute to the greater performance detriments with lower oxygenation levels (range of 70–75% arterial oxygen saturation), which is likely regulated by differences in the partial pressure of arterial oxygen ($P_{a,O2}$) rather than changes in arterial content or hemoglobin concentration (Horstman et al., 1980; Calbet et al., 2002; Lundby and Damsgaard, 2006).

Limitations of Cerebral Oxygenation

Greater decreases in prefrontal cortex deoxygenation (Δ[HHb]) were demonstrated at 3800 m. Cerebral changes in oxygenation were limited (lower) at both 2000 and 3800 m, in comparison to the 400 m condition. Furthermore, greater changes in [tHb], a well-known indicator of regional blood volume and perfusion (Van Beekvelt et al., 2001; Faiss et al., 2013), and Δ[Hbdiff] were seen at the end of the test (i.e., exhaustion, last 20% of sprints) with increased changes at both 2000 and 3800 m for Δ[Hbdiff]. These data together illustrate the oxygen delivery regulation of the brain.

Previous research has indicated that in hypoxic conditions there is a greater diffusive limitation due to a lower gradient of the partial pressure of oxygen (Wagner, 1993). In hypoxia, the brain adjusts for a lower arterial oxygen content by increasing the extraction of oxygen (Gonzalez-Alonso et al., 2004; Rasmussen et al., 2007) and increasing cerebral blood flow at rest (Lassen, 1959; Willie et al., 2012). While during exercise, there are the additional factors of hypocapnia and increased arterial blood pressure which contribute to the impact of oxygenation and brain function in hypoxia (Curtelin et al., 2017). Curtelin et al. (2017) found that the priority is placed on maintaining cerebral oxygen delivery even though there is also a need to regulate cerebral blood flow to avoid an excessive increase in blood pressure. During sprint exercise in hypoxia, the combination of increased mean arterial pressure and cardiac output increases perfusion of the muscle tissue while simultaneously challenging the brain with the risk of hyperperfusion (Bill and Linder, 1976; Deegan et al., 2010). As demonstrated in the present study, there were greater changes in the perfusion (Δ[tHb]) in the end of the test, which was likely tolerated despite higher perfusion pressure and reduced partial pressure of arterial carbon dioxide ($P_{a,CO2}$) during sprint exercise in severe hypoxia in order to protect brain function in low oxygen conditions with the counter-risk of increased hemodynamic injury (Curtelin et al., 2017). This change was mostly present in the normoxic condition, however, there was a main effect difference at the end of exercise. Further, this finding may provide insight to the reason for exercise cessation. Moreover, the evidence of previous research showing a 66% decrease in cerebral $P_{a,O2}$ with acute hypoxia cannot be ignored (Calbet et al., 2009). In fact, it was found that the low $P_{a,O2}$ may be as low as 10 Torr in some areas of the brain (Calbet et al., 2003). This low $P_{a,O2}$ would suggest there is a low pressure gradient, however it remains unknown about the diffusion distance and the possible diffusive limitation of oxygen delivery to the brain at altitude (Hornbein et al., 1989; Dunn et al., 2016). There is likely a coupling between arterial blood pressure and perfusion pressure (gradient between arterial and venous blood pressure) related to pressure, flow, and resistance to determine the mechanisms of the diffusion limitation of cerebral oxygen delivery during exhaustive exercise in hypoxia.

The results of the present study confirm that exercise performance in hypoxia can continue despite limited peripheral convective oxygen delivery, with the continuation of performance due to a functional reserve in muscle oxygen diffusing capacity (Calbet et al., 2015b). Meanwhile, the brain increases blood flow to account for decreased arterial oxygen content and to maintain oxygen delivery to preserve the brain's function (central motor output) and thus task continuation despite the progression of central fatigue (Amann et al., 2007; Curtelin et al., 2017). Progressive changes continue until an underlying threshold is reached where the pressure of increased perfusion of the brain surpasses a critical threshold and likely leads to exercise cessation due to increased mean arterial pressure and hyperperfusion. However, these mechanisms are speculative and therefore further research is warranted.

Limitations of the Study

Researchers acknowledge that the current results are influenced by an elevation of skin blood flow during exercise, which is due to the nature of the device placed on the surface of the skin (Subudhi et al., 2007). Therefore, it is probable that the current results would underestimate tissue deoxygenation. Moreover, it is likely that the changes found would be greater in these variables and would not alter the conclusions made here. The difference between hemoglobin and myoglobin is not known with the use of the NIRS, it is therefore not possible to distinguish the differences between blood-muscle oxygen transport in the current study. Furthermore, the reliability of probe placement was minimized with permanent markings and picture identification, however, there is day-to-day variation in these parameters of about 8.0–9.4% (Kishi et al., 2003; Kolb et al., 2004). It is assumed that [O_2Hb] and [HHb] are in existing equilibrium where an increase in one simultaneously decreases the other. This assumption is based on maintaining [tHb] constant. As most of the body's arterial blood volume is oxygenated, [tHb] is closely related to [O_2Hb] if the consumption of oxygen remains constant, which can be reflected by HHb concentration (Van Beekvelt et al., 2001; Subudhi et al., 2007). During exercise, these variables are in constant flux and for that reason, the NIRS measurements should be considered with scrutiny. In addition, the test-retest reliability of the blood flow measurements from the femoral artery conducted pre- and post-RSAT have not been previously reported, thus it is important to consider in interpretation of these results. As this study involved non-invasive measurements as part of a larger protocol, there is a lack of data corresponding to changes in blood gases, blood pressure, and cerebral blood flow which would certainly improve the interpretation of these results.

CONCLUSION

In summary, this study confirms that performance in hypoxia is limited by continually decreasing convective oxygen delivery while exercise can continue despite maximal peripheral deoxygenation. The limitation of peripheral oxygenation was not linear across varying hypoxic conditions possibly indicating a threshold at which point oxygen delivery has a stronger effect on the periphery between 2000 and 3800 m. There may be a cerebral autoregulation that increases cerebral perfusion at exhaustion to account for decreased arterial oxygen content (as shown across all conditions) and allow for task continuation (greater cerebral deoxygenation at 3800 m in comparison to 400 and 2000 m).

AUTHOR CONTRIBUTIONS

All authors listed have made substantial, direct, and intellectual contributions to this work. In addition, all authors have approved this work for publication.

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Variations in Hypoxia Impairs Muscle Oxygenation and Performance during Simulated Team-Sport Running

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Purpose: To quantify the effect of acute hypoxia on muscle oxygenation and power during simulated team-sport running.

Methods: Seven individuals performed repeated and single sprint efforts, embedded in a simulated team-sport running protocol, on a non-motorized treadmill in normoxia (sea-level), and acute normobaric hypoxia (simulated altitudes of 2,000 and 3,000 m). Mean and peak power was quantified during all sprints and repeated sprints. Mean total work, heart rate, blood oxygen saturation, and quadriceps muscle deoxyhaemoglobin concentration (assessed via near-infrared spectroscopy) were measured over the entire protocol. A linear mixed model was used to estimate performance and physiological effects across each half of the protocol. Changes were expressed in standardized units for assessment of magnitude. Uncertainty in the changes was expressed as a 90% confidence interval and interpreted via non-clinical magnitude-based inference.

Results: Mean total work was reduced at 2,000 m (−10%, 90% confidence limits ±6%) and 3,000 m (−15%, ±5%) compared with sea-level. Mean heart rate was reduced at 3,000 m compared with 2,000 m (−3, ±3 min^{-1}) and sea-level (−3, ±3 min^{-1}). Blood oxygen saturation was lower at 2,000 m (−8, ±3%) and 3,000 m (−15, ±2%) compared with sea-level. Sprint mean power across the entire protocol was reduced at 3,000 m compared with 2,000 m (−12%, ±3%) and sea-level (−14%, ±4%). In the second half of the protocol, sprint mean power was reduced at 3,000 m compared to 2,000 m (−6%, ±4%). Sprint mean peak power across the entire protocol was lowered at 2,000 m (−10%, ±6%) and 3,000 m (−16%, ±6%) compared with sea-level. During repeated sprints, mean peak power was lower at 2,000 m (−8%, ±7%) and 3,000 m (−8%, ±7%) compared with sea-level. In the second half of the protocol, repeated sprint mean power was reduced at 3,000 m compared to 2,000 m (−7%, ±5%) and sea-level (−9%, ±5%). Quadriceps muscle deoxyhaemoglobin concentration was lowered at 3,000 m compared to 2,000 m (−10, ±12%) and sea-level (−11, ±12%).

Conclusions: Simulated team-sport running is impaired at 3,000 m compared to 2,000 m and sea-level, likely due to a higher muscle deoxygenation.

Keywords: altitude, non-motorized treadmill, near-infrared spectroscopy, repeated sprints

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INTRODUCTION

Athletic performance is reduced at altitude, including elevations of 580 m (Gore et al., 1997). Team-sport matches are contested at a range of terrestrial altitudes including 1,600 m (Johannesburg, South Africa) and 3,600 m (La Paz, Bolivia). The impact of moderate to high altitude on junior-elite soccer performance has recently been investigated (Aughey et al., 2013; Garvican et al., 2013; Buchheit et al., 2015). At 1,600 m, total distance and high-speed running were reduced by 9 and 15%, respectively (Garvican et al., 2013). At 3,600 m, the 5-min peak period of high-speed running and total distance covered was lowered by 20.8 and 9.1%, respectively (Aughey et al., 2013). Acclimatization to, and native residence at, 3,600 m does not protect against the detrimental effects of altitude on team-sport athlete match running (Aughey et al., 2013). Altitude therefore has a substantial impact on running performance during junior-elite soccer matches. Understanding the magnitude and impact of altitude exposure on team-sport match running may assist in optimizing athlete physical preparation for playing at altitude.

The influence of altitude on team-sport athlete match running is typically investigated through repeated sprint laboratory studies. The capacity to perform sprint efforts is considered important for scoring during team-sport matches (Faude et al., 2012). Laboratory repeated sprint tests, performed on a non-motorized treadmill, are generally designed to replicate periods of match running and allow measurement of an athlete's capacity to resist fatigue. In team-sport athletes, peak power during three sets of nine repeated sprints, of 4-s in duration, was maintained at a simulated altitude of 3,000 m but not 4,000 m (Goods et al., 2014). The peak speed performed by untrained individuals is reduced during 10 6-s repeated-sprints in varying hypoxic conditions compared to normoxia (Bowtell et al., 2014). The distance covered by amateur team-sport athletes during the final two sets of four 4-s repeated sprints was also reduced in normobaric hypoxia compared to normoxia (Morrison et al., 2015). Simulated altitude therefore has a detrimental effect on laboratory repeated sprint performance. However, assessing the impact of titrated altitude during controlled laboratory studies does not replicate the chaotic and complex nature of team-sport matches (Sirotic and Coutts, 2007). During repeated sprint laboratory studies, with known exercise and rest periods, participants may plan their physical output. In matches, team-sport athletes perform low-speed activity interspersed with high-intensity movement. Exercise intensity may be regulated dependent upon an individual's ability to perform high-intensity activity (Castagna and Abt, 2003). Under conditions of environmental stress, athletes may moderate this low-speed activity to preserve the capacity to perform hard efforts, including repeated sprints (Aughey et al., 2013, 2014). Laboratory studies designed to mimic environmental stress, such as simulating altitude, should therefore include periods of lower-intensity work interspersed with repeated sprint efforts to simulate the running performed by team-sport athletes during matches.

The impact of altitude on physical performance during a simulated team-sport movement protocol was recently examined (Aldous et al., 2015). Total and high-speed distance covered by team-sport athletes during a 90-min protocol was reduced by 4% at 1,000 m simulated altitude compared to sea-level. The distance covered in each of the two 45-min halves was 10% lower at altitude compared to sea-level, with total sprint distance also reduced in the final 15 min of the protocol. Perceived exertion across the entire protocol was also 7% lower at sea-level compared to altitude. Since team-sport matches are contested at a range of altitudes around the world, the dose-response relationship of varying altitudes on simulated match running should be explored. The subsequent impact of these altitudes on the physiological determinants of team-sport running should also be examined.

Physiological determinants including neural, ionic and metabolic factors underpin the capacity to perform repeated sprint efforts (Billaut et al., 2012). Moderately trained individuals with a high maximal oxygen uptake ($\dot{V}O_{2max}$) may have an increased resynthesis rate of phosphocreatine (PCr), a vital substrate during intermittent high-intensity exercise (Harris et al., 1976). Attained exclusively via aerobic sources, the resynthesis of PCr is dependent upon O_2 and can be completely suppressed via limb blood flow occlusion (Harris et al., 1976). Muscle O_2 kinetics and PCr resynthesis are also tightly linked (Kime et al., 2003). A high perfusion and reoxygenation rate of the active musculature during recovery periods, assessed via near-infrared spectroscopy (NIRS), is paramount to reproduce a high performance in a subsequent bout (Ufland et al., 2012). The delivery of O_2 is also highly sensitive to manipulations of environmental O_2 and compromised at altitude, which may contribute to impairment during repeated-sprint exercise (Billaut and Buchheit, 2013). The capacity of skeletal muscle to be reoxygenated during 30 s of passive rest between repeated sprint running efforts is reduced by up to 33% in hypoxia (Bowtell et al., 2014), although NIRS has not been utilized to assess muscle reoxygenation during simulated team-sport running at varying altitudes. Since PCr resynthesis is a main factor of performance during repeated sprint efforts, the slower repletion of this substrate at altitude may be detrimental to team-sport running performance. Therefore, the aim of the present study was to quantify the effect of acute, titrated hypoxia on physical output and physiological responses during simulated team-sport running.

METHODS

Participants

Seven (six males and one female) individuals (age 27.0 ± 6.6 years; height 179.7 ± 6.1 cm; $\dot{V}O_{2max}$ 59.5 ± 5.1 mL.kg^{-1}.min^{-1}; body mass 75.1 ± 10.2 kg at commencement of study, [mean ± Standard Deviation (SD)] provided written informed consent to participate in the study. Participants were from team-sport plus endurance backgrounds and trained at least twice per week. The study was approved by the university Human Research Ethics Committee and conformed to the declaration of Helsinki. Participants were sea-level natives and had not visited >2,000 m altitude for more than 24 h in the 4 months prior to participating in the study.

Study Design

A single-blind randomized design, with altitude counter-balanced, was employed. All testing was conducted within a 23.92 m^2 environmental exercise laboratory. During their first visit, participants completed a $\dot{V}O_{2max}$ test to assess maximal aerobic power. The test consisted of 3 × 4 min periods of incremental running before speed was maintained and gradient increased by 1% each minute until volitional exhaustion (Robertson et al., 2010). Participants then returned for a separate familiarization session, involving a 5 min warm-up at 100 W on a cycle ergometer (Velotron, Seattle, USA) followed by four maximal 4 s sprints, each separated by 14 s of passive recovery, on the non-motorized treadmill (Woodway Force, Waukesha, WI, USA). Individual speed ranges for the simulated team-sport movement protocol, described below, were established from the maximal speed attained during six sprint efforts on the non-motorized treadmill (Sirotic and Coutts, 2008). Participants then completed a modified (condensed to 13 min) version of the team-sport running protocol. The familiarization session, 1 week after the preliminary visit, involved completion of the entire simulated team-sport movement protocol and NIRS measurements. Participants then completed a testing session at sea-level followed by two randomized, counter-balanced sessions under titrated hypoxic conditions. Each session involved a standardized 5 min cycling warm-up at 100 W and completion of the simulated team-sport running protocol. Normobaric hypoxia was created through nitrogen injection into the environmental exercise laboratory. The F_iO_2 was ∼0.163 and ∼0.143, simulating altitudes of 2,000 m and 3,000 m, respectively. Simulated altitudes were based on the definition of low to moderate altitude (Bartsch et al., 2008). Each session was conducted at similar times of the day for each participant to limit influence of the circadian rhythm on sprint-induced neuromuscular fatigue (Giacomoni et al., 2006). All sessions were separated by 1 week. Participants were encouraged to refrain from physical activity and caffeine in the 24 h prior to each session.

Simulated Team-Sport Running Protocol

Participants were secured onto the non-motorized treadmill using a manufacturer provided belt. The belt connected to a horizontal force transducer, adjusted for each participant by measuring and reproducing the displacement between the anterior superior illiac spine and the belt, as per Serpiello et al. (2011). Participants were encouraged to keep this consistent throughout all sessions. The force transducers were calibrated, before each participant's session, according to the manufacturer's guidelines.

The 26.4 min protocol (Zois et al., 2013) required participants to move through six individually-established speed zones, displayed in **Figure 1**. The total percentage of each activity was; standing (33%), walking (23%), jogging (23%), running (13%), fast running (4%), and sprinting (4%). The protocol included three repeated-sprint tests (set 1 and 3; four 4 s sprints and set 2; two 4 s sprints, all interspersed with 14 s passive recovery) and 10 single sprint efforts, all 4 s in duration. Visual (screen above the non-motorized treadmill) and verbal (strong 3-2-1 countdown to the upcoming change in activity) instructions on the target speed were given. Visual instructions included a red line on the screen, or each participant's individual target speed, and a green line indicating their actual speed. Participants were required to match their current speed with the target speed as closely as possible for all of the six speed zones. Verbal cues were given by the same operator to reduce variability. Data was sampled at 50 Hz and exported to customized software to analyze the mean total work completed across each half of the protocol. The first half was activity performed with the first 12-min of the protocol and the second half was activity post the 13-min mark. Minute 12 to 13 was a passive resting period and consequently removed from analysis. The average mean and peak power was calculated for all sprints in each half. The average mean and peak power was also calculated for each of the first and third set of repeated sprints. All sprints were defined as the first occurrence of speed at 1 $m{\cdot}s^{-1}$ and from this point, a 4 s period was subsequently calculated (Serpiello et al., 2011). Peak power was determined as the highest single value recorded during a sprint. The coefficient of variation (CV) for mean power and mean peak power was 3.3 and 4.8%, respectively.

Physiological Measures

Blood oxygen saturation (S_pO_2) was continuously measured at the earlobe by pulse oximetry (SPO Medical equipment, Israel) and collected for each minute across the entire protocol. Average S_pO_2 data was analyzed for the first and second half of the protocol. Heart rate (HR) was recorded each minute of the protocol and averaged for the first plus second half. Rating of perceived exertion (RPE) was collected each minute, across both halves, via the Borg 6–20 scale. The mean RPE for each half of the protocol was analyzed.

Oxygenation of the vastus lateralis was monitored during all trials using a NIRS device (Oxymon MkIII, Artinis, The Netherlands). The emitter and detector probe pair, with an optode distance of 4 cm, were attached using black plastic spacers. Probes were placed on the muscle belly (∼15 cm above the proximal border of the patella and 5 cm lateral to the midline of the thigh) of the dominant lower limb and protected from external light using black bandages. A modified form of the Beer–Lambert Law calculated micromolar changes in tissue oxyhaemoglobin [O_2Hb] and deoxyhaemoglobin [HHb] across time using received optical densities from two continuous wavelengths of NIR light (763 and 855 nM). The differential optical path-length factor was 4.95 (Billaut and Buchheit, 2013). Skinfold thickness (5.7 ± 1.4 mm) was less than half of the emitter-detector distance in each participant.

Analysis concentrated on Δ[HHb], as it is primarily independent of total hemoglobin (De Blasi et al., 1993). Maximal [HHb] was obtained post-exercise via pressure cuff inflation (∼300 mmHg), at the base of the thigh until the increased Δ[HHb] plateaued. The leg ischemia was performed whilst standing to mimic the sprint position. All Δ[HHb] values presented are expressed as a percentage of the amplitude of change between baseline and maximal [HHb] values. Data was acquired at 10 Hz throughout the entire protocol and averaged for each minute of the protocol. Data was also obtained during

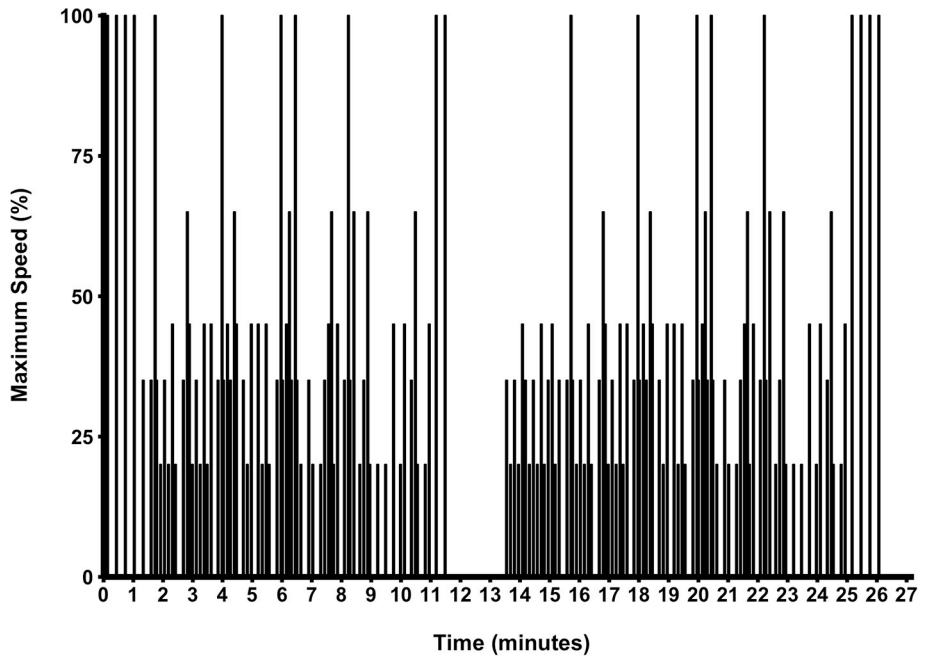

FIGURE 1 | The simulated team-sport running protocol.

the recovery periods from repeated sprint efforts, within the first and third set of repeated sprints.

Statistical Analysis

Separate analyses were performed for each of the measures derived from the non-motorized and physiological variables. Each analysis was performed with the same mixed model using the general linear mixed-model procedure (Proc Mixed) in the Statistical Analysis System (version 9.4, SAS Institute, Cary NC). The fixed effects in the model were altitude (three levels: sea-level, 2,000, 3,000 m), the interaction of altitude with a dummy variable representing the second half of the testing session (to estimate mean change between the first and second half, representing mean fatigue in the second half), and a dummy variable representing the second trial at altitude (to adjust the altitude effects to the first trial at altitude). The following random effects were specified: participant identity (to allow for repeated measurement on participants) and its interaction with the second-half dummy variable (with unstructured covariance, to allow for individual second-half means, representing individual differences in fatigue); participant identity interacted with trial identity (to allow for the repeated measurement on participants within trials); participant identity interacted with a dummy variable representing non-sea level (to estimate individual responses to altitude); and the residual (representing error of measurement from half to half). There were insufficient observations to allow successful specification of different but correlated individual responses to the two altitudes.

All measures, except for the physiological measures, were log-transformed before analysis then back-transformed to express the changes in percent units. The changes were also expressed in standardized units for assessment of magnitude. Standardization was performed by dividing the change score of the log-transformed measure by the between-participant standard deviation derived from the random effects for participant and its interaction with trial identity; this between-participant standard deviation represents the differences between participant free of residual (measurement) error. Uncertainty in the changes was expressed as 90% confidence intervals and interpreted via the non-clinical magnitude-based inference approach (Hopkins et al., 2009). Standardized changes of 0.20, 0.60, 1.20, 2.0, and 4.0 were thresholds for small, moderate, large, very large, and extremely large effects, respectively (Hopkins et al., 2009). When the confidence interval for a change included small positive and negative effects, the change was deemed unclear. For clear effects, the likelihood that the true effect was substantial was indicated with the following scale: *possibly* (25–75%), *likely* (75 to <95%), *very likely* (95–99.5%), and *most likely* (>99.5%).

RESULTS

The mean and ± standard deviation of mean heart rate, vastus lateralis deoxyhemoglobin, total work, oxygen saturation, and rating of perceived exertion per half of the simulated team-sport running protocol at each altitude are presented in **Figure 2**. The mean ± standard deviation for mean and peak power during all sprint efforts, per level of altitude and each half, is presented in **Figure 3**. The mean ± standard deviation for Vastus lateralis [HHb] during recovery periods and average blood oxygenation saturation per half is presented in **Figure 4**. The magnitude of change in performance and physiological variables, across

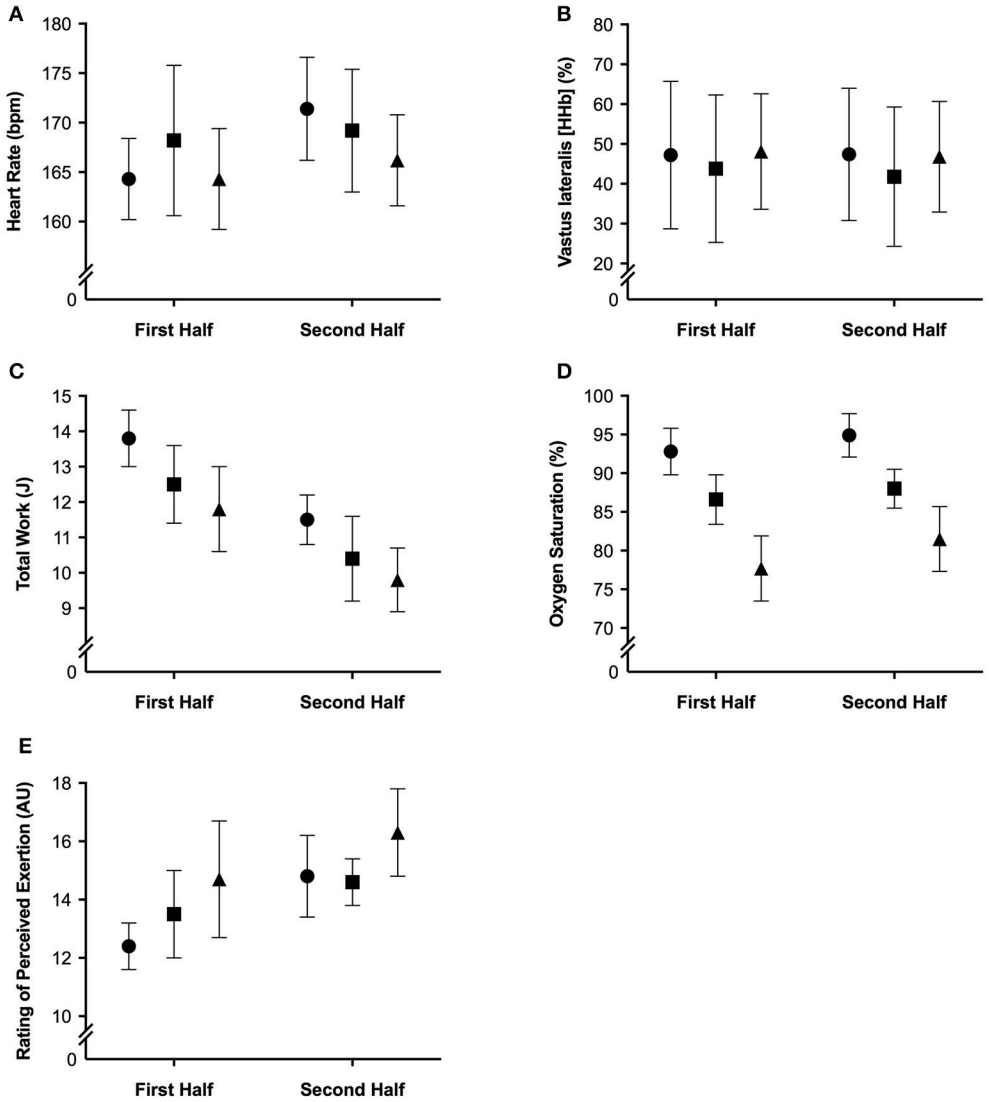

FIGURE 2 | The mean ± standard deviation for (A) heart rate; **(B)** vastus lateralis deoxyhemoglobin; **(C)** total work; **(D)** oxygen saturation, and **(E)** rating of perceived exertion per half of the simulated team-sport running protocol. Circles: 0 m; rectangles: 2,000 m; triangles: 3,000 m.

the entire protocol, per half, and compared across levels of altitude are presented in **Table 1**. The magnitude of change in performance and physiological variables, during repeated sprints, per half, and compared across levels of altitude are presented in **Table 2**. The magnitude of change in performance and physiological variables at altitude, across the second half of the protocol, and during the final trial, are presented in **Table 3**. The magnitude of change in performance and physiological variables at altitude, during repeated-sprints only, across the second half of the protocol, and during the final trial, are presented in **Table 4**.

DISCUSSION

Mean total work was substantially reduced during simulated team-sport movement at 2,000 and 3,000 m compared to sea-level. Mean total work was also most likely reduced in the second half of the protocol compared to the first at sea-level, 2,000 and 3,000 m. During sprint efforts across the simulated running protocol, mean and peak power was reduced at 3,000 m compared to sea-level. During repeated-sprints in the second half of the protocol, mean power was possibly reduced at 3,000 m, with no clear differences at 2,000 m or sea-level. During recovery from repeated-sprint efforts, vastus lateralis [HHb] was possibly reduced at 3,000 m compared to SL. This study is the first to use linear mixed modeling to examine the effect of titrated altitude on muscle oxygenation during simulated team-sport movement. This statistical approach provides detail on the percentage effects of fixed and individual factors, superior to other commonly used statistical methods, that may contribute to a decrement in performance at altitude.

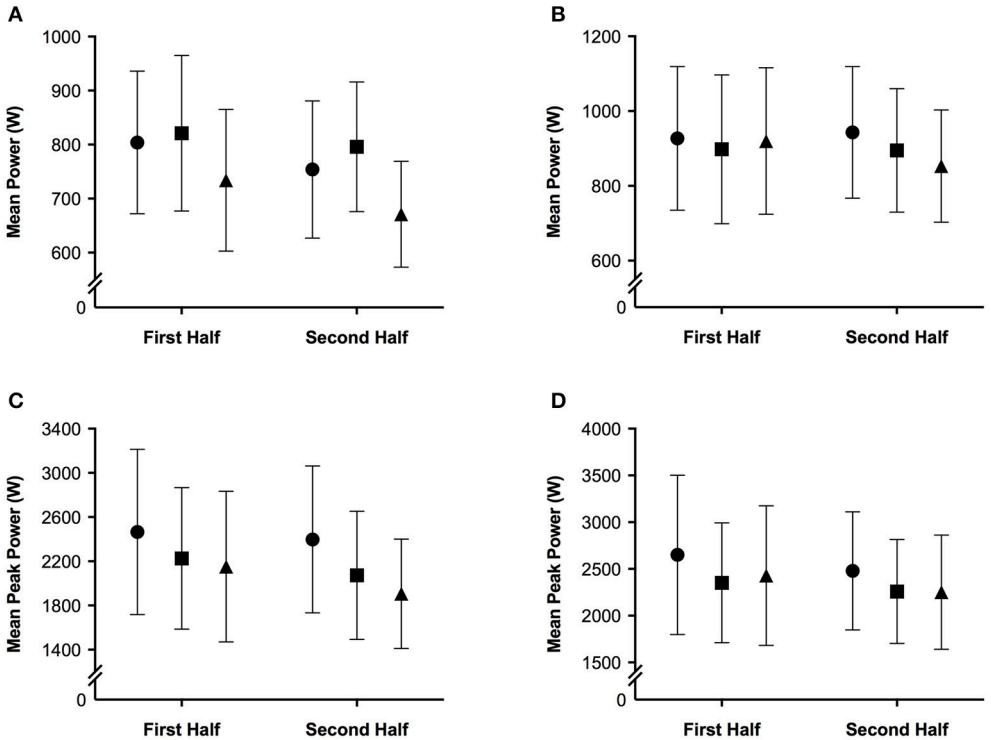

FIGURE 3 | The mean ± standard deviation for (A) mean power during all sprint efforts; **(B)** mean power during repeated sprints only; **(C)** mean peak power during all sprint efforts; **(D)** mean peak power during repeated sprints only per half of the simulated team-sport running protocol. Circles: 0 m; rectangles: 2,000 m; triangles: 3,000 m.

During repeated-sprint efforts, vastus lateralis [HHb] was likely higher in the second half at 2,000 m compared SL. This reoxygenation impairment is in agreement with the increased [HHb] (relative to normoxia) and performance decrement observed during recovery of ten 10 s sprints under 0.13 F_iO_2 Billaut and Buchheit (2013) during occlusion-free conditions, it is still debated whether the primary determinant of muscle post-exercise reoxygenation is muscle O_2 delivery or local utilization (Hamaoka et al., 1998; Kime et al., 2003). In the present study, the incomplete muscle reoxygenation observed in the second half at 3,000 m was likely related to limited muscle O_2 delivery post-sprint (Billaut and Buchheit, 2013). The resynthesis of PCr is derived solely from aerobic sources (Harris et al., 1976). In hypoxia, aerobic capacity is considerably impaired (Gore et al., 1996) and an inverse relationship between O_2 supply and PCr resynthesis rate has been established *in vitro* (Idstrom et al., 1985). In normoxia, PCr stores recovered to only 45% of resting concentrations after five 6 s sprints interspersed with 24-s passive recovery (Dawson et al., 1997). The current study employed 4-s repeated-sprints interspersed with 14-s recovery. Between RS efforts, PCr was likely not fully resynthesized due to a hypoxia-induced decrease in O_2 availability to working musculature, with S_pO_2 7.7 and 14.6% lower across the entire protocol at 2,000 and 3,000 m, respectively. Metabolic recovery and subsequent sprint performance was likely compromised, although the resynthesis of PCr was not measured in the present study. Impaired O_2 transport in hypoxia likely increased the contribution of anaerobic glycolysis to exercise metabolism, (Morales-Alamo et al., 2012) accelerating the development of peripheral muscle fatigue and decreased central motor drive (Amann and Dempsey, 2008). Cerebral oxygenation and electromyography data would provide a detailed understanding of the acute fatigue mechanisms during repeated-sprint sets at titrated altitudes.

Mean total work performed during the simulated team-sport movement was considerably diminished at 2,000 and 3,000 m compared to SL. This is in agreement with the large reduction in total work during three sets of (9 × 4 s) repeated running sprints at 2,000 and 3,000 m simulated altitude (Goods et al., 2014). In the present study, total work was reduced by ∼5.1% per 1,000 m rise in altitude, lower than the ∼7% per 1,000 m decline during 5 min time-trial performance (Clark et al., 2007), and 14% per 1,000 m decrement in a time-to-exhaustion (total duration of 308 s) test (Wehrlin and Hallen, 2006). The smaller reduction in the present study may be attributed to variation in the length and type of exercise. In contrast with the relatively short-duration, all-out time-trial (Clark et al., 2007), and time-to-exhaustion (Wehrlin and Hallen, 2006) tests, the 26.4 min protocol included lower-intensity movements and recovery, closer to replicating team-sport running. The recovery afforded by these lower-intensity periods (and associated lower energy expenditure) likely assisted the maintenance of repeated-sprint performance.

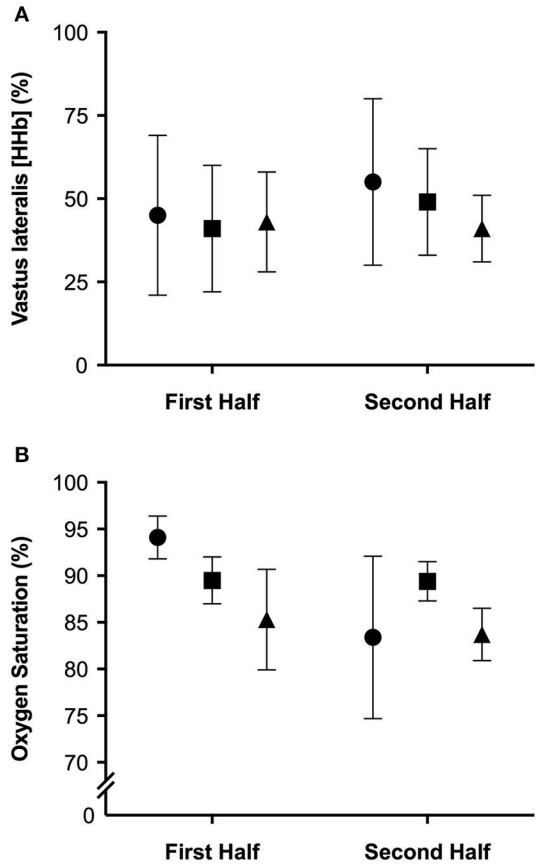

FIGURE 4 | The mean ± standard deviation values for (A) vastus lateralis deoxyhemoglobin during recovery from repeated sprints; **(B)** oxygen saturation during repeated sprints, per half. Circles: 0 m; rectangles: 2,000 m; triangles: 3,000 m.

Although, an active recovery from short repeated cycling sprints may disrupt the resynthesis of PCr (Spencer et al., 2008), our findings are reinforced by data collected during an elite youth soccer match at 1,600 m (Garvican et al., 2013). High-speed running was impaired by 15% and low-speed running plus total distance were reduced by 9 and 8%, respectively (Garvican et al., 2013). During a match at 3,600 m, peak 5 min running distance by elite youth soccer athletes was also reduced by 13–16% (Aughey et al., 2013). Collectively, this data demonstrates the negative impact of altitude on team-sport running performance.

Participants may have paced their effort, via self-selecting a lower intensity under hypoxia, between repeated-sprint sets in an attempt to preserve the ability to perform high-intensity efforts. Whilst the majority of pacing research focuses on continuous events (de Koning et al., 2011), pacing is also evident during repeated-sprint exercise. Knowledge of an exercise end-point can determine athletic performance. During a deception trial (where participants were uninformed on the number of sprints to be completed), less muscle mass was recruited and mechanical work lowered, presumably to avoid excessive metabolic disturbances, and muscle fatigue (Billaut et al., 2011). In the present study, participants were aware of the duration and number of sprints to be completed. Participants were also visually guided on the speed to be performed throughout the entire protocol, including the sprints.Participants may have been slower to reach target velocities or maintain these velocities during sprint efforts embedded in the protocol, in the aim of maintaining repeated-sprints located at the end of the protocol. The preservation of repeated-sprint efforts at 2,000 m are in agreement with the maintenance of repeated-running sprints in hypoxia from 12 to 21% O_2 (Bowtell et al., 2014). Fatigue index (calculated as the percentage decrease in peak speed from fastest to slowest speed) was greater when breathing 12% O_2 (corresponding altitude of 4,345 m; Bowtell et al., 2014). At a simulated altitude of 4,000 m, mean power output was also reduced during the first set of 9 × 4 s running repeated-sprint (Goods et al., 2014). Mean power was also lowered in subsequent sets at 2,000, 3,000, and 4,000 m (Goods et al., 2014), emphasizing the critical role of O_2 in PCr resynthesis and repeated-sprint performance (Harris et al., 1976).

Due to the chaotic nature of competition, athletes are unaware of the number of high-intensity bouts to be completed. Many high-intensity actions are performed when the ball is in possession, dispute or an opportunity to score arises (Faude et al., 2012). Thus, team-sport athletes are less able to modulate lower speed activities to preserve sprint capacity. This is in opposition with laboratory-based repeated-sprint studies, where participants are typically aware of the number of efforts to be completed. In the present study, participants were informed about lower-intensity activities and despite constant visual information on the speed to be maintained, they were unable or chose not to do so in hypoxia. At 3,000 m simulated altitude, the hypoxic stress appears to negatively influence physical output compared to 2,000 m. Coaching and conditioning staff should be aware of the negative impact of 3,000 m altitude upon team-sport running performance. Athletes may need to be interchanged or substituted when competing at 3,000 m, to minimize the detrimental impact of high altitude and allow sufficient recovery from high-speed running. Training at 3,000 m prior to competing at altitude may negate the detrimental effects of hypoxia on team-sport running, although this remains to be investigated. The short protocol duration of 26.4 min, in the present study, was designed to simulate an intensified period of team-sport activity. A 30 min simulated team-sport running protocol is a reliable tool for assessing and monitoring the physiological and performance of team-sport activity (Sirotic and Coutts, 2008). Increasing the protocol duration of the current study to 52.8 or 79.2 min could more closely replicate the duration of a team-sport match although it is unclear what impact this would have on test-retest variation, particularly at hypoxia. Future studies should investigate the impact of hypoxia on a longer simulated running protocol and use a linear-mixed modeling approach to estimate the individual and fixed factors that contribute to performance. Future studies investigating individual responses to simulated altitude should also monitor the training loads, sleep, nutrition, and recovery of participants during performance testing.

TABLE 1 | The difference in magnitude of change between levels of altitude on mean total work, heart rate, vastus lateralis deoxygenation, rating of perceived exertion, blood oxygenation saturation, mean sprint power, and mean sprint peak power across the entire protocol.

	Comparison (m)	Difference across entire protocol	Difference in second half of protocol
Mean total work (%)	2,000–0	**−10.3, ±5.5; ↓ *** **	**0.1, ±4.7**
	3,000–0	**−14.9, ±5.2; ↓ **** **	**−0.3, ±4.7**
	3,000–2,000	**−5.1, ±2.7; ↓ *** **	**−0.4, ±4.7**
Mean HR (bpm)	2,000–0	0.4, ±2.5	−3.6, ±2.3
	3,000–0	−2.7, ±2.4; ↓ **	**−3.4, ±2.4; ↓ ** **
	3,000–2,000	**−3.1, ±2.5; ↓ ** **	**0.2, ±2.5**
Mean Δ[HHb] (%)	2,000–0	−3.9, ±9.1	−2.2, ±3.3; $\leftrightarrow^{00}$
	3,000–0	0.7, ±8.8	−1.5, ±3.3; $\leftrightarrow^{00}$
	3,000–2,000	4.5, ±7.3; ↑ *	0.7, ±3.3; $\leftrightarrow^{00}$
Mean RPE (AU)	2,000–0	0.1, ±1.6	−1.1, ±0.8; ↓ **
	3,000–0	1.7, ±1.4; ↑ **	−0.5, ±0.8
	3,000–2,000	1.5, ±1.3; ↑ **	0.6, ±0.8; ↑ *
Mean S_pO_2(%)	2,000–0	**−7.7, ±2.5; ↓ **** **	1.4, ±1.4; ↑ **
	3,000–0	**−14.6, ±2.4; ↓ **** **	**2.3, ±1.3; ↑ *** **
	3,000–2,000	**−7.0, ±1.7; ↓ **** **	0.9, ±1.0; ↑ *
Sprint mean Power (%)	2,000–0	−1.9, ±4.9; $\leftrightarrow^{00}$	**3.8, ±6.8; ↑ ***
	3,000–0 m	**−13.8, ±4.3; ↓ **** **	−1.9, ±3.6; $\leftrightarrow^{00}$
	3,000–2,000	**−12.0, ±2.9; ↓ **** **	**−5.5, ±3.5; ↓ ** **
Sprint mean peak Power (%)	2,000–0	**−10.1, ±6.4**; ↓ ** **	**−4.9, ±6.1; ↓ ***
	3,000–0	**−15.6, ±6.0; ↓ *** **	**−8.7, ±5.8**; ↓ *
	3,000–2,000	**−6.1, ±3.7; ↓ ***	**−4.0, ±6.1; $\leftrightarrow^{00}$**

*Values are presented as raw or % change in mean, ±90% CI or ±99% CI; Direction of response: positive ↑ negative ↓ trivial ↔; Symbols denote: *possibly, **likely, ***very likely, and ****most likely chance of the true effect exceeding a small (0.2) effect. Trivial changes indicated by ^{0}. m, meters; HR, heart rate; Δ[HHb], change in deoxyhemoglobin; RPE, rating of perceived exertion; S_pO_2, blood oxygenation saturation. Bold text indicates change in the mean at the 99% confidence interval (CI).*

TABLE 2 | The difference in magnitude of change between levels of altitude on mean and peak power, vastus lateralis deoxygenation during recovery periods, and blood oxygenation saturation during repeated sprints only.

	Comparison	Difference across entire protocol	Difference in second half of protocol
Repeated sprint mean power (%)	2,000–0	**−4.6, ±3.3; ↓ ***	−1.7, ±5.3;$\leftrightarrow^{00}$
	3,000–0	**−5.5, ±3.2; ↓ ***	**−8.8, ±4.9; ↓ ** **
	3,000–2,000	**−1.0, ±1.8;** $\leftrightarrow^{0000}$	**−7.2, ±5.0; ↓ ***
Repeated sprint mean peak power (%)	2,000–0	**−7.8, ±7.3; ↓ ***	1.3, ±12.6
	3,000–0	**−7.5, ±7.2; ↓ ***	−2.2, ±12.1
	3,000–2,000	−0.3, ±5.1; $\leftrightarrow^{0000}$	−3.5, ±12.0
Mean recovery HHb (%)	2,000–0	−3.5, ±11.6	−1.3, ±12.3
	3,000–0	−6.9, ±10.7; **↓ ***	−11.0, ±12.3; ↓ **
	3,000–2,000	−3.4, ±12.2	−9.7, ±12.3; ↓ **
Mean set S_pO_2(%)	2,000–0	−0.4, ±6.8	**15.0, ±3.7; ↑ **** **
	3,000–0	−5.3, ±6.5; ↓ **	**13.1, ±3.6; ↑ **** **
	3,000–2,000	−4.9, ±6.4	−1.9, ±3.5

*Values are presented as % change in mean, ± 90% CI or ±99% CI; Direction of response: positive ↑ negative↓ trivial ↔; Symbols denote: *possibly, **likely, ***very likely, and ****most likely chance of the true effect exceeding a small (0.2) effect. Trivial changes indicated by ^{0}. m, meters; Δ[HHb], change in deoxyhemoglobin; S_pO_2, blood oxygenation saturation. Bold text indicates change in the mean at the 99% confidence interval (CI).*

TABLE 3 | The difference in magnitude of change at altitude on mean total work, heart rate, vastus lateralis deoxygenation, rating of perceived exertion, blood oxygenation saturation, mean sprint power, and mean sprint peak power across the second half of the protocol and during the final trial, irrespective of altitude.

	Altitude (m)	Difference in second half of protocol	Difference in final trial
Mean total work (%)	0	**−16.9, ±3.5; ↓ ******	−0.5, ±2.9
	2,000	**−16.8, ±3.5; ↓ ******	
	3,000	**−17.2, ±3.5; ↓ ******	
Mean HR (bpm)	0	**4.5, ±2.7; ↑ ****	−0.2, ±2.4
	2,000	0.7, ±2.5; $\leftrightarrow^{00}$	
	3,000	0.9, ±2.6	
Mean Δ[HHb] (%)	0	0.2, ±2.8	−1.1, ±7.2
	2,000	−2.0, ±2.7; ↓ *	
	3,000	−1.3, ±2.7; ↓ *	
Mean RPE (AU)	0	**2.2, ±1.1; ↑ ***	0.2, ±1.3
	2,000	**1.1, ±1.1;** $\leftrightarrow^{00}$	
	3,000	**1.7, ±1.1; ↑ ***	
Mean S_pO_2(%)	0	**0.5, ±1.1;** $\leftrightarrow^{000}$	−0.6, ±1.7; ↑ *
	2,000	**1.9, ±0.8; ↑ *****	
	3,000	**2.8, ±0.8; ↑ ******	
Sprint mean power (%)	0	**−6.3, ±2.9; ↓ ******	**10.9, ±3.7; ↑ ******
	2,000	−2.8, ±3.0; ↓ **	
	3,000	**−8.1, ±2.8; ↓ ******	
Sprint mean peak power (%)	0	−1.8, ±8.4	−2.3, ±6.2
	2,000	−6.5, ±8.0	
	3,000	−10.3, ±7.6; ↓ ***	

*Values are presented as raw or % change in mean, ±90% CI or ± 99% CI; Direction of response: positive ↑ negative↓ trivial ↔; Symbols denote: *possibly, **likely, ***very likely, and ****most likely chance of the true effect exceeding a small (0.2) effect. Trivial changes indicated by ^{0}. m, meters; HR, heart rate; Δ[HHb], change in deoxyhemoglobin; RPE, rating of perceived exertion; S_pO_2, blood oxygenation saturation. Bold text indicates change in the mean at the 99% confidence interval (CI).*

TABLE 4 | The difference in magnitude of change at altitude on mean and peak power, vastus lateralis deoxygenation during recovery periods, and blood oxygenation saturation during repeated sprints only and during the final trial, irrespective of altitude.

	Altitude (m)	Difference in second half of protocol	Difference in final trial
Repeated sprint mean power (%)	0	2.4, ±8.3	**0.8, ±1.8;** $\leftrightarrow^{0000}$
	2,000	0.6, ±8.2	
	3,000	−6.6, ±7.6; ↓ *	
Repeated sprint mean peak power (%)	0	−4.3, ±10.6; ↓ *	**−3.0, ±4.9;** $\leftrightarrow^{00}$
	2,000	−3.1, ±10.8	
	3,000	−6.4, ±10.4; ↓ *	
Mean recovery HHb (%)	0	**9.8, ±7.9; ↑ ****	−2.0, ±12.2
	2,000	8.5, ±7.9; **↑ ****	
	3,000	−1.3, ±7.9	
Mean set S_pO_2(%)	0	**−14.7, ±2.6; ↓ ******	−2.1, ±6.4
	2,000	0.3, ±2.3	
	3,000	−1.5, ±2.6; ↓ *	

*Values are presented as % change in mean, ±90% CI or ± 99% CI; Direction of response: positive ↑ negative↓ trivial ↔; Symbols denote: *possibly, **likely, ***very likely, and ****most likely chance of the true effect exceeding a small (0.2) effect. Trivial changes indicated by ^{0}. m, meters; Δ[HHb], change in deoxyhemoglobin; S_pO_2, blood oxygenation saturation. Bold text indicates change in the mean at the 99% confidence interval (CI).*

CONCLUSION

During simulated team-sport movement, repeated-sprint and single-sprint efforts are compromised at 3,000 m altitude, possibly due to limited muscle O_2 availability during recovery periods. To overcome this decrement, participants reduced their total work completed despite strong verbal encouragement. Whilst this strategy may have assisted in largely maintaining repeated-sprint and single-sprint efforts at 2,000 m, the elevated physiological demands at 3,000 m may have been overwhelming. Consequently, repeated-sprint and single-sprint performance was subsequently impaired. Under hypoxia, the decrement in work completed during intermittent running is lower than previously reported for time-trial and time-to-exhaustion tests.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of ethical guidelines, as declared by the Victoria University High Risk Ethics Committee, with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Victoria University High Risk Ethics Committee.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: FB and RA. Performed experiments: AS, FB, RR, MV, and RA. Analyzed data: AS. Interpreted results of research: AS, RA, RR, MV, FB, WH. Drafted manuscript and prepared tables/figures: AS. Edited, critically revised paper, and approved final version of manuscript: AS, RR, MV, FB, WH, and RA.

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High Altitude Increases Alteration in Maximal Torque but Not in Rapid Torque Development in Knee Extensors after Repeated Treadmill Sprinting

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We assessed knee extensor neuromuscular adjustments following repeated treadmill sprints in different normobaric hypoxia conditions, with special reference to rapid muscle torque production capacity. Thirteen team- and racquet-sport athletes undertook 8 × 5-s "all-out" sprints (passive recovery = 25 s) on a non-motorized treadmill in normoxia (NM; FiO_2 = 20.9%), at low (LA; FiO_2 = 16.8%) and high (HA; FiO_2 = 13.3%) normobaric hypoxia (simulated altitudes of ∼1800 m and ∼3600 m, respectively). Explosive (∼1 s; "fast" instruction) and maximal (∼5 s; "hard" instruction) voluntary isometric contractions (MVC) of the knee extensors (KE), with concurrent electromyographic (EMG) activity recordings of the *vastus lateralis* (VL) and *rectus femoris* (RF) muscles, were performed before and 1-min post-exercise. Rate of torque development (RTD) and EMG (i.e., Root Mean Square or RMS) rise from 0 to 30, −50, −100, and −200 ms were recorded, and were also normalized to maximal torque and EMG values, respectively. Distance covered during the first 5-s sprint was similar ($P > 0.05$) in all conditions. A larger ($P < 0.05$) sprint decrement score and a shorter ($P < 0.05$) cumulated distance covered over the eight sprints occurred in HA (−8 ± 4% and 178 ± 11 m) but not in LA (−7 ± 3% and 181 ± 10 m) compared to NM (−5 ± 2% and 183 ± 9 m). Compared to NM (−9 ± 7%), a larger ($P < 0.05$) reduction in MVC torque occurred post-exercise in HA (−14 ± 9%) but not in LA (-12 ± 7%), with no difference between NM and LA ($P > 0.05$). Irrespectively of condition ($P > 0.05$), peak RTD (−6 ± 11%; $P < 0.05$), and normalized peak RMS activity for VL (−8 ± 11%; $P = 0.07$) and RF (−14 ± 11%; $P < 0.01$) muscles were reduced post-exercise, whereas reductions ($P < 0.05$) in absolute RTD occurred within the 0–100 (−8 ± 9%) and 0–200 ms (−10 ± 8%) epochs after contraction onset. After normalization to MVC torque, there was no difference in RTD values. Additionally, the EMG rise for VL muscle was similar ($P > 0.05$), whereas it increased ($P < 0.05$) for RF muscle during all epochs post-exercise, independently of the conditions. In summary, alteration in repeated-sprint ability and post-exercise MVC decrease were greater at high altitude than in normoxia or at low altitude. However, the post-exercise alterations in RTD were similar between normoxia and low-to-high hypoxia.

Keywords: repeated-sprint ability, hypoxia, rapid torque development, neural drive, voluntary force production

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INTRODUCTION

Intense physical efforts performed at or near maximal speed and the ability to recover from it are important markers of successful in-game performance in high-intensity, intermittent sports (Spencer et al., 2005). Team- and racquet-sport players implement training methods, based on the repetition of maximal efforts in normoxia (e.g., repeated sprints, Bishop et al., 2011) or in hypoxia (e.g., repeated sprints in hypoxia; Brocherie et al., 2015a), for eliciting neuromuscular adaptations (e.g., enhanced muscle oxygenation and activation responses) and improving cardiovascular and metabolic function, in turn maximizing their physical performance. Although the physiological responses and potential metabolic limiting factors (i.e., limitations in energy supply, metabolite accumulation) associated with the completion of one repeated-sprint set have largely been described, there is comparatively less data on the neuromuscular consequences (Girard et al., 2011).

Neuromuscular fatigue is an exercise-induced reduction in the maximal isometric voluntary contraction (MVC) force/torque or power of a muscle group, which potentially involves alterations at any levels from the brain to skeletal muscles (Gandevia, 2001). Over the past decade, an increasing number of studies have quantified neuromuscular fatigue following repeated running (Perrey et al., 2010) or cycling (Racinais et al., 2007; Billaut et al., 2013; Girard et al., 2013; Hureau et al., 2014) sprints by comparing pre- to post-sprint values of force/torque, voluntary activation, electromyogram (EMG), and twitch responses. Recent studies assessed the neuromuscular function during the actual repeated-sprint sets (Goodall et al., 2015; Pearcey et al., 2015). With regard to MVC, however, it is important to note that maximal force/torque production capacity is most often obtained when exceeding 300 ms following contraction onset (Thorstensson et al., 1976). This is contrasting to the characteristics of muscular contraction occurring in several sporting events, where muscle force needs to be developed in less than 250 ms (e.g., sprints: Kuitunen et al., 2002; jumps: Luhtanen and Komi, 1979). Therefore, the ability to rapidly generate force/torque, i.e., the rate of force/torque development (RTD) within the initial (i.e., <250 ms) phase of an MVC which in turn correlates with sprint performance (Tillin et al., 2013), likely constitutes a more functional outcome measure (Girard and Millet, 2009). Therefore, RTD as a surrogate for explosive strength should be assessed for a better understanding of the acute neuromuscular adjustments to repeated sprinting.

Repeated-sprint ability is more altered at high (>3000–3500 m or FiO_2 below 13–14%) than lower altitudes, either normoxia or low-to-moderate (<3000 m or FiO_2 above 14%) altitude (Bowtell et al., 2014; Goods et al., 2014). Not only acute hypoxic exposure decreases convective O_2 transport (i.e., reduction in arterial O_2 saturation values or SpO_2), but also challenges multiple regulatory systems by increasing cardiorespiratory (i.e., higher heart rate, minute ventilation, O_2 debt), metabolic (i.e., slower muscle re-oxygenation responses) and/or neuromuscular (i.e., incomplete muscle activation) requirements during sprinting or subsequent recovery periods (Balsom et al., 1994; Billaut et al., 2013; Bowtell et al., 2014). Compared to normoxia, the completion of a repeated-sprint cycling protocol (15 × 5-s efforts, 25-s rest) in high hypoxia (FiO_2 = 14%) led to ∼8% lower total mechanical work as a result of impaired muscle activation, which was also accompanied by ∼6% lower post-exercise MVC (Billaut et al., 2013). However, these authors did not include any measure of explosive strength in their study.

To our knowledge, only one study has investigated the effect of repeated sprinting (i.e., 10 × 6-s "all out" cycling sprints, followed, after 6 min of passive rest, by 5 × 6-s sprints; recoveries = 30 s) performance on post-exercise alterations in rapid muscle torque production capacity of the knee extensors (KE) (Girard et al., 2013). MVC (−12%) and RTD (−15 to −26% from the 0–30 to 0–200 ms epochs after contraction onset) decreased during brief (i.e., 5 s) contractions after (i.e., 3 min) the repeated-sprint exercise. From this report, however, it is not entirely clear to which extent differences in RTD actually resulted from maximal voluntary strength adjustments, since RTD results were not normalized to MVC torque. Hence, whereas MVC torque losses following prolonged match-play tennis accounted for the dampened RTD values (Girard et al., 2014), fatigue induced by 10 sets of voluntary maximal explosive contractions exerted a more rapid and pronounced effect (particularly during the initial 50 ms of contraction) on explosive strength than MVC torque (Buckthorpe et al., 2014). Consequently, although repeated sprinting decreases RTD (Girard et al., 2013), the question of whether RTD at early and/or late intervals is more pronouncedly impaired than MVC has not been specifically addressed.

The analysis of EMG amplitude throughout the rising force-time curve can reveal how voluntary neural drive to skeletal muscle underlies the post-exercise decreased RTD. Hence, the rate of muscle activation is related to muscle shortening velocity (Nelson, 1996), a factor directly influencing RTD (Harridge et al., 1996). Furthermore, the determinants of explosive force production appear to change throughout the rising force-time curve (Folland et al., 2014) and fatigue may differentially affect the development of force throughout the time course of an explosive contraction. After repeated sprinting, non-significant reductions in *vastus lateralis* (VL) Root Mean Square (RMS) activities have been shown to accompany deteriorated RTD values (Girard et al., 2013). However, because the delay between exercise termination and post-exercise neuromuscular testing was 3 min in the aforementioned study, any meaningful changes in the central nervous performance may have already recovered. Furthermore, it has not been investigated if the early and later RTD time intervals (and associated rate of EMG activity rise) are in fact modified differently by performing repeated-sprint in various hypoxia severity levels. This question of the alteration in explosive strength post-hypoxic exposure/training is of high practical relevance in team- and racquet-sports but remains unclear: Hence, while countermovement jump performance increased to a similar extent after repeated-sprint training in normoxia vs. hypoxia (Brocherie et al., 2015a), movement velocity and power during the execution of a force-velocity in bench-press are improved when exposed to hypobaric vs. normobaric hypoxia in reference to normoxia (Feriche et al., 2014).

The aim of this study was therefore to assess the effects of repeated sprinting in different levels of normobaric hypoxia on the alterations in RTD and neuromuscular activity of KE. Given that there is "*extraordinarily little that changes with regard to maximal force-generating capacity with acute hypoxia*" (Perrey and Rupp, 2009), it was hypothesized that the already-known decrease in repeated-sprint ability (i.e., lower fatigue resistance) under high hypoxic conditions would not be associated with more pronounced alterations in explosive force production (i.e., RTD) compared to normoxia or low hypoxia.

METHODS

Participants

Thirteen male recreational team- (i.e., football, rugby, basketball) and racket- (i.e., tennis, squash) sport athletes (Mean ± SD: 31.2 ± 4.8 years; 178.4 ± 6.6 cm; 74.3 ± 8.2 kg) participated in the study. All participants were born and raised at <1000 m and had not traveled to elevations >1000 m in the 3 months prior to investigation. They gave their informed, written consent preceding the commencement of the experiment. Experimental protocol was conducted according to the Declaration of Helsinki for use of Human Subjects and approved by the Ethics Committee of *Shafallah Medical Genetics Center*.

Study Design

Elements have previously been reported in Girard et al. (2015a). About a week prior to testing, participants undertook a complete preliminary session where they performed short (<5 s) treadmill sprints at increasing intensities wearing a facemask for habituation (i.e., with the hypoxic system turned off), with full recovery and until being comfortable with the running technique required (which generally necessitated 7–10 trials). Then they performed three maximal 5-s single sprints, separated by 2 min of passive recovery, and after 5 min of rest, the repeated-sprint exercise test in full. All of them satisfied the criteria of having a coefficient of variation < 2.2% for distance covered across three successive trials (Girard et al., 2015b). Strong verbal encouragement was given during all maximal efforts. Participants were also thoroughly familiarized with the neuromuscular function assessment protocol (see *Neuromuscular Function*) until they felt accustomed with the equipment (i.e., coefficient of variation in three successive KE trials for peak RFD and maximal torque with "fast" and "hard" instructions lower than 5 and 3%, respectively).

Participants reported to the laboratory (well-ventilated at a constant temperature of ∼25°C and 40% relative humidity) on three different occasions (∼1 h; counterbalanced randomized crossover design in double-blind fashion) at least 3–4 days apart to complete an experimental session. This involved performing a repeated-sprint running protocol on a sprint treadmill (ADAL3D-WR, Medical Development – HEF Tecmachine, Andrézieux-Bouthéon, France), allowing participants to produce realistic acceleration and high running velocities (Morin et al., 2010). Participants performed their trials at the same time of the day (± 1 h) and wore similar sports gear (running shoes, short, and T-shirt). They were instructed to maintain their normal diet (i.e., avoiding any nutritional supplements or alcohol consumption), sleeping (i.e., ≥7 h/night) and training (i.e., avoiding vigorous exercise 24 h before every trial) habits during the 1–2 weeks period of testing to prevent any possible interference on their sprinting abilities. Participants were instructed to drink 4–6 mL of water per kilogram of body mass every 2.5 h on the day before each experimental session to ensure euhydration at the start of exercise. They were permitted to drink *ad libitum* during the warm-up procedure.

Experimental Protocol

Upon arrival on testing days, participants were instrumented and pre-exercise (Pre-tests) neuromuscular function assessment (see *Neuromuscular Function*) was conducted in normoxia. Thereafter, they completed a running warm-up (i.e., on the sprint treadmill with participants breathing ambient air) consisting of 5 min of running at 10 km.h^{-1}, followed by 10 min of sprint-specific muscular warm-up exercises [i.e., 3 × (skipping, high knee, butt-kick, high heels for ∼10 s with 30-s walking in between), followed by 3 × (3 steps accelerations at a subjective "sense of effort" of 7, 8, and 9 on a modified Borg 10 scale), then by 2 × (3-s sprints at a subjective "sense of effort" of 8 and 9)] (Christian et al., 2014). Afterwards, three maximal 5-s single sprints, separated by 2 min of passive recovery, were completed. After a facemask connected to a portable hypoxic generator (Altitrainer, SMTEC SA, Nyon, Switzerland) had been attached on participants, they were allowed 5-min of free cool-down prior to the repeated-sprint protocol. This exercise consisted of performing eight, 5-s "all-out" sprints interspersed with 25 s of passive rest and was randomly conducted in normoxia (NM; FiO_2 = 20.9%), in low and high simulated altitudes (normobaric hypoxia) of ∼1800 m (LA; FiO_2 = 16.8%) and ∼3600 m (HA; FiO_2 = 13.3%), respectively. Normobaric hypoxia was obtained by mixing nitrogen into ambient air under control of FiO_2. During recovery periods, participants stood quietly on the treadmill. Repeated-sprint ability was assessed from covered distance data using three scores: the largest (i.e., during the first sprint in all cases) distance ran, the cumulated distance covered over the eight sprints (i.e., sum of the eight sprints) and the sprint decrement score [i.e., ([cumulated distance/(largest distance × 8)]–1) × 100] (Girard et al., 2011). Finally, the neuromuscular function assessment was repeated (Post-tests) in normoxia (i.e., participants took off the facemask 25 s after completion of the last sprint) and was started exactly 1 min after the repeated-sprint exercise protocol ended.

Responses to Exercise

Heart rate and SpO_2 were monitored and estimated, respectively, via a Polar transmitter-receiver (Wearlink T-31, Polar Electro Oy, Kempele, Finland) and non-invasive pulse oximetry using a finger probe (Palmsat, 2500, NONIN Medical Inc., Plymouth, MI, USA). Together with heart rate and SpO_2, ratings of perceived exertion were recorded using the Borg 6–20 scale (i.e., 6 = no exertion at all, 20 = maximal exertion) exactly 10 s following each sprint (i.e., peak values likely to be obtained). Additionally, SpO_2 was recorded between before the warm-up

and 4 min after the last sprint. These time points corresponded to the end of pre- and post-tests.

Neuromuscular Function

Neuromuscular test sessions began by the completion of three successful MVCs, all brief (∼5 s) and separated by ≥30 s of rest, with a twitch delivered over the isometric plateau. Participants were instructed to increase torque production over a 1-s period, hold it for 3–4 s and then relax before completing the next contraction. Thereafter, participants were instructed to perform "explosive" MVCs (separated by ≥20 s). During all brief MVCs trials the participants were carefully instructed to contract "as fast as possible" for ∼1 s from a fully relaxed state, in an attempt to achieve at least 90% of their MVC torque. Participants were asked to avoid any countermovement before torque onset; i.e., they were reminded not to flex the knee immediately prior to KE. They were strongly encouraged with verbal feedback and a visual display of the torque production. Contractions that had any discernable countermovement or pre-tension (i.e., change of baseline torque of >1.5 Nm during the 100 ms before contraction onset; Girard et al., 2014) were discarded and another attempt was made. To provide biofeedback on whether a countermovement had occurred, the resting torque level was displayed on a sensitive scale. The slope of the torque–time curve (10 ms time constant) was displayed throughout testing and the peak slope was used to provide visual performance feedback to participants after each contraction. Pre-tests assessment was preceded by a warm-up consisting of 10 isometric contractions of ∼3–5 s in duration interspaced with ∼10–20 s of recovery. Contraction intensity was progressively self-adjusted by the participant to attain maximal torque in the last three contractions.

Recordings

Torque Measurements

KE torque was measured with participants seated upright on a custom-built adjustable chair with the hips and knees flexed at 90°. Restraining straps placed across the chest and hips secured the participants in the chair to prevent extraneous movement, while the dynamometer (Captels, St Mathieu de Treviers, France) was attached 3–5 cm above the tip of the lateral malleoli. During all contractions the torque signals were amplified, sent through an A/D board and sampled at 2000 Hz by commercially available hardware and software (MP35 and BSL Pro Version 3.6.7, Biopac Systems Inc., Santa Barbara, USA).

Electromyography

The EMG activity of the VL and *rectus femoris* (RF) muscles was recorded via bipolar Ag/AgCl electrodes (Ambu Blue sensor T, Ambu A/S, Denmark; diameter = 9 mm; inter-distance electrode = 30 mm) fixed longitudinally over the muscle bellies. The reference electrode was attached to the right wrist. Low impedance between the two electrodes was obtained by abrading the skin with emery paper and cleaning with alcohol. The position of the electrodes was marked for consistent placement. EMG signals were amplified (gain = 1000), filtered (band-width frequency 30–500 Hz) and recorded (sampling frequency = 2000 Hz) by commercially available hardware (Biopac MP35, systems Inc., Santa Barbara, CA) and software (Acqknowledge 3.6.7, Biopac Systems Inc., Santa Barbara, CA).

Motor Nerve Stimulation

Femoral nerve stimulations (400 V, rectangular pulse of 0.2 ms) were delivered by a high-voltage stimulator (Digitimer DS7AH; Digitimer, Hertfordshire, UK) via a cathode electrode (diameter of 5 mm) placed in the inguinal crease and an anode (5 × 10 cm; Medicompex, SA, Ecublens, Switzerland) in the gluteal fold. The intensity of stimulation was determined during the familiarization test session using a passive isometric recruitment curve (Racinais et al., 2013). Briefly, the stimulation intensity was increased by 10-mA increments until a maximal peak twitch torque was achieved and then a further increased by 50% to ensure constant supramaximal stimulation throughout the protocol.

Data Analysis

All analyses were performed using Spike 2 Software (Cambridge Electronic Design, Cambridge, UK). The MVC torque was defined as the maximum value recorded for 1 s when the torque had reached a plateau (before the superimposed twitch), and the RMS of the EMG activity was computed during the same 1-s period (RMS_{MAX}). Similarly, the peak-to-peak amplitude of superimposed maximum compound action potential (M-wave) responses was measured for each agonist muscle, and RMS_{MAX} was divided by M-wave to give a ratio RMS_{MAX}/M-wave.

The contractile RTD (expressed as $Nm.s^{-1}$) was derived from the "explosive" MVC measurements, as the average slope of the initial time phase of the torque-time curve at 0–30, 0–50, 0–100, and 0–200 ms, relative to the onset of contraction (Aagaard et al., 2002; Suetta et al., 2004; Thorlund et al., 2009) using a custom written program (Spike 2 Software, Cambridge Electronic Design, Cambridge, UK). The onset of muscle contraction was defined as the time point at which the torque curve exceeded baseline by >4.5 Nm, corresponding to ∼2.5% of MVC torque values (Andersen et al., 2010). The peak RTD was defined as the peak Δtorque/Δtime achieved during the initial 200 ms of the isometric contraction (de Oliveira et al., 2013; Girard et al., 2014). In addition, the rate of muscle activation (expressed as $mV.s^{-1}$) was measured as the raw RMS activity increase obtained at similar time intervals relative to onset integration (i.e., activity). The onset of EMG integration was shifted 50 ms before the onset of contraction to account for the presence of electromechanical delay (Aagaard et al., 2002; Girard et al., 2013). The RTD and EMG rise were also normalized relative to maximal MVC torque (%MVC) and maximal EMG activity (%RMS_{MAX}/M-wave). The mean over three trials was used for further analysis for each parameter.

Statistical Analysis

Values are expressed as means ± SD. Two-way repeated-measures ANOVAs [Time (Pre-tests vs. Post-tests) × Condition (NM, LA vs. HA)] were used to compare torque and muscle activation data for each time window (0–30, 0–50, 0–100, and 0–200 ms) independently for absolute and relative changes.

Outcome variables were tested using Mauchly's procedure for sphericity. Whenever the data violated the assumption of sphericity, *P*-values and adjusted degrees of freedom based on Greenhouse-Geisser correction were reported instead. Where significant effects were established, pairwise differences were identified using the Bonferroni *post-hoc* analysis procedure adjusted for multiple comparisons. For each ANOVA, partial eta-squared was calculated as measures of effect size. Values of 0.01, 0.06, and above 0.14 were considered as small, medium, and large, respectively. All statistical calculations were performed using SPSS statistical software V.21.0 (IBM Corp., Armonk, NY, USA). The significance level was set at $P < 0.05$.

RESULTS

Repeated-Sprint Ability and Responses to Exercise

Distance covered during the first 5-s sprint was similar (24.2 ± 1.4, 24.5 ± 1.5, and 24.4 ± 1.7 m for NM, LA, and HA, respectively; $P > 0.05$) across conditions (**Figure 1**). In reference to sprint 1, distance covered decreased from sprint 2 onwards ($P < 0.001$), independently of the condition ($P = 0.324$). The averaged values of distance covered for sprints 1–8 were lower in HA (22.3 ± 1.3 m) compared to NM (22.9 ± 1.2 m; $P = 0.044$) but not LA (22.7 ± 1.3 m; $P = 0.183$), with also no difference between NM and LA ($P = 0.710$). A larger sprint decrement score occurred in HA (−7.8 ± 3.6%) vs. NM (−5.3 ± 1.9%; $P = 0.015$) but not LA (−6.3 ± 3.5%; $P = 0.060$), with also no difference between NM and LA ($P = 0.237$). Compared to NM (183.2 ± 9.3 m), the cumulated distance covered over the eight sprints was shorter in HA (178.5 ± 10.7 m; $P = 0.014$) but not in LA (181.4 ± 10.3 m; $P = 0.056$), with also no difference between NM and LA ($P = 0.240$).

Whereas, it did not change in NM (96.8 ± 0.8 vs. 96.0 ± 1.6%; $P = 0.455$), SpO_2 values decreased from the first to the last sprint in LA (95.0 ± 2.0 vs. 89.7 ± 4.0%; $P = 0.050$) and HA (88.8 ± 2.5 vs. 81.8 ± 4.5%; $P = 0.001$) (**Table 1**). Compared to pre-tests (96.9 ± 0.4%), SpO_2 values were not different among conditions during post-tests after completion of the repeated-sprint ability protocol (96.2 ± 0.5%; all conditions pooled, $P > 0.05$). Heart rate (NM: 150 ± 15 vs. 176 ± 12 bpm; LA: 154 ± 9 vs. 176 ± 9 bpm; HA: 154 ± 12 vs. 174 ± 13 bpm, all $P < 0.001$) and ratings of perceived exertion (NM: 11.8 ± 1.4 vs. 18.5 ± 1.2 points; LA: 11.3 ± 1.4 vs. 18.1 ± 1.6 points; HA: 11.5 ± 1.8 vs. 19.1 ± 0.8 points, all $P < 0.001$) increased from sprint 1 to sprint 8, irrespective of the environmental condition ($P = 0.256$). Higher ratings of perceived exertion values were recorded for the average of eight sprints in HA (15.8 ± 0.3 points) vs. LA (15.2 ± 0.3 points; $P = 0.006$), but not NM (15.5 ± 0.3 points; $P = 0.452$).

Maximal Strength

Compared to NM (−9 ± 7%), a larger ($P < 0.05$) reduction in MVC torque occurred from pre- to post-exercise in HA (−14 ± 9%; $P = 0.021$) but not in LA (−12 ± 7%; $P = 0.270$), with

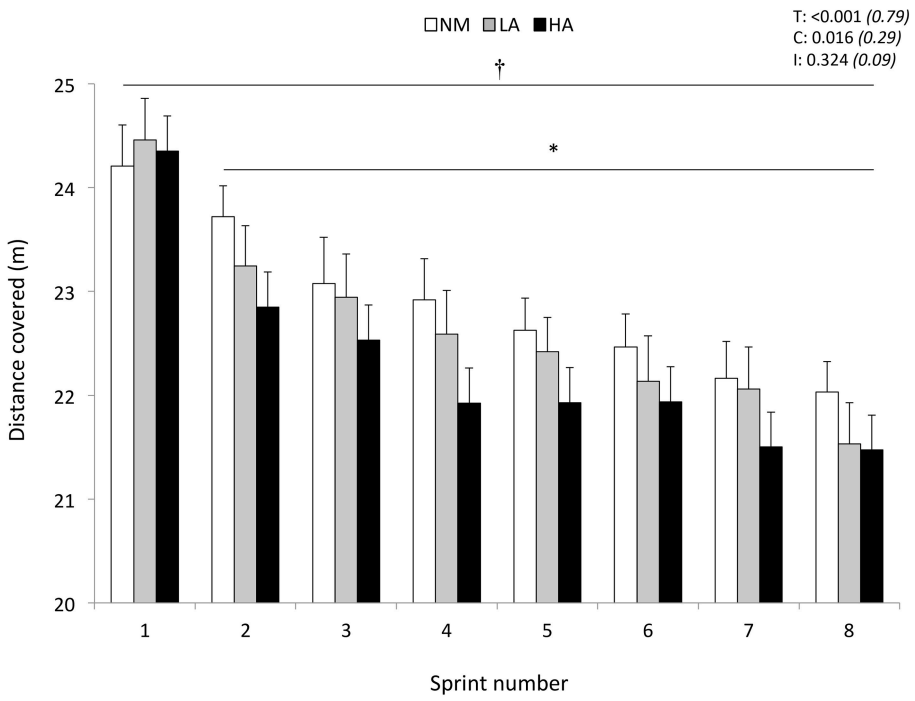

FIGURE 1 | Distance covered during the repeated-sprint ability test. Data are presented in normoxia (NM; $FiO_2 = 20.9\%$), at low (LA; $FiO_2 = 16.8\%$), and high (HA; $FiO_2 = 13.3\%$) normobaric hypoxia. Values are mean ± SD ($n = 13$). T, C, and I respectively refer to ANOVA main effects of time, condition, and interaction between these two factors with *P*-value and partial eta-squared in parentheses. *Significantly different from sprint 1 (all conditions pooled), $P < 0.05$. †NM different from HA (all sprints pooled), $P < 0.05$.

also no differences between LA and HA ($P = 0.340$) (**Table 2**). During MVCs, raw EMG signals of both VL and RF muscles were lower at post- relative to pre-, with no difference between conditions. Peak-to-peak M-wave amplitudes for both VL and RF muscles did not change. A global reduction of the RMS_{MAX}/M-wave ratio occurred from pre- to post-exercise for the RF ($-14 \pm 11\%$; $P = 0.002$), while failing to reach statistical significance for the VL ($-8 \pm 11\%$; $P = 0.075$).

Rapid Muscle Characteristics

Peak RTD (all conditions pooled: $-6 \pm 11\%$; $P = 0.031$) was significantly reduced from pre- to post-exercise (**Table 2**). Reduction in RTD (absolute values) occurred within the 0–100 ($-8 \pm 9\%$; $P = 0.011$) and 0–200 ms ($-10 \pm 8\%$; $P < 0.001$) epochs after contraction onset, independent of the condition ($P > 0.23$; **Figure 2**). No differences in RTD (relative values) were observed after normalization to MVC torque ($P > 0.197$; **Figure 3**). Furthermore, the relative rates of EMG rise for VL muscle for any epochs throughout the experimental protocol were not different between conditions ($P > 0.49$) and there were no interaction ($P > 0.42$) or time effects ($P > 0.12$) (**Figure 4**). The rate of EMG rise for RF muscle increased during the periods 0–30 ($+22 \pm 26\%$; $P = 0.006$), 0–50 ($+25 \pm 28\%$; $P = 0.002$), 0–100 ($+27 \pm 27\%$; $P < 0.001$), and 0–200 ms ($+23 \pm 2\%$; $P = 0.002$) post-exercise, independently of the condition ($P > 0.23$; **Figure 5**).

DISCUSSION

Repeated-Sprint Performance and Responses to Exercise

Distance ran during the first 5-s sprint was similar in all conditions, as an enhanced anaerobic energy release can compensate for the reduced aerobic ATP production during short maximal efforts in hypoxic conditions (Calbet et al., 2003). Compared to NM, a larger sprint decrement score and a shorter cumulated distance covered from sprint 1 to 8 occurred in HA, but not in LA, with also no difference between NM and LA. Consistent with previous repeated sprinting literature (Bowtell et al., 2014), our data therefore show that performance fatigability was significantly exacerbated relative to NM only under our severer hypoxic condition. The question of whether the same is true when completing identical repeated sprinting protocol at natural altitude (i.e., hypobaric hypoxia), known to induce severer physiological responses (Millet et al., 2012) and eventually larger neuromuscular alterations, has not been specifically addressed. Hence, the decrease in air density upon ascent to terrestrial altitude reduces air resistance, which is likely to decrease the energy cost of running at high velocities, and thereby improve single sprint performance (Levine et al., 2008). When sprints are repeated, however, hypobaric hypoxia would induce higher work of breathing responses and more detrimental neuromuscular consequences than exposure to gas mixtures lowering FiO_2. Despite lower SpO_2 values in the severer hypoxic condition, it is interesting to observe in this study that sensations that regulated the integrity of the performer (ratings of perceived exertion or perceived fatigability) and associated heart rate responses did not differ between SL and HA.

Neuromuscular Parameters during Maximal Contractions

Along with lower distance covered during the repeated-sprint test, post-exercise reduction in maximal KE torque, as measured from brief MVCs, was ∼5% larger in HA (−14%) compared to NM (−9%). This later result is in line with a previous study where the decrease in KE MVC torque under hypoxia ($FiO_2 = 14\%$) was 6% larger in reference to normoxia after the completion

TABLE 1 | Changes in responses to exercise during the repeated-sprint ability test in normoxia (NM; $FiO_2 = 20.9\%$), under low (LA; $FiO_2 = 16.8\%$) and high (HA; $FiO_2 = 13.3\%$) normobaric hypoxia.

Variables	Sprint number								ANOVA *(Partial eta-squared)*
	1	2	3	4	5	6	7	8	
SpO_2 (%)									
NM	96.8 ± 0.8	96.4 ± 1.9	96.3 ± 1.9	95.8 ± 2.0	96.2 ± 2.1	95.8 ± 2.0	95.8 ± 1.5	96.0 ± 1.6	T < 0.001 *(0.60)*
LA#	95.0 ± 2.0	94.3 ± 3.2	92.4 ± 3.4*	91.3 ± 2.9*	91.5 ± 3.0*	91.5 ± 3.0	89.6 ± 4.4*	89.7 ± 4.0*	C < 0.001 *(0.85)*
HA#,†	88.8 ± 2.5	86.1 ± 4.5	84.5 ± 4.1*	84.0 ± 4.9*	83.6 ± 5.2*	83.2 ± 4.6*	82.5 ± 5.3*	81.8 ± 4.5*	I = 0.016 *(0.24)*
HR (bpm)									
NM	150 ± 15	164 ± 13*	169 ± 13*	171 ± 12*	174 ± 12*	175 ± 11*	175 ± 11*	176 ± 12*	T < 0.001 *(0.92)*
LA	154 ± 9	166 ± 11*	172 ± 9*	175 ± 10*	176 ± 10*	176 ± 11*	176 ± 10*	176 ± 9*	C = 0.427 *(0.07)*
HA	154 ± 12	166 ± 15*	171 ± 13*	174 ± 14*	174 ± 13*	175 ± 12*	174 ± 12*	174 ± 13*	I = 0.187 *(0.12)*
RPE (POINTS)									
NM	11.8 ± 1.4	13.0 ± 1.1*	14.3 ± 1.4*	15.5 ± 1.5*	16.4 ± 1.7*	17.1 ± 1.6*	17.7 ± 1.5*	18.5 ± 1.2*	T < 0.001 *(0.95)*
LA	11.3 ± 1.4	12.7 ± 1.1*	13.8 ± 1.2*	15.1 ± 1.1*	16.1 ± 1.2*	17.0 ± 1.5*	17.6 ± 1.4*	18.1 ± 1.6*	C = 0.034 *(0.25)*
HA†	11.5 ± 1.8	13.0 ± 1.6*	14.4 ± 1.6*	15.7 ± 1.6*	16.7 ± 1.4*	17.5 ± 1.3*	18.4 ± 1.1*	19.1 ± 0.8*	I = 0.256 *(0.10)*

Mean ± SD (n = 13).

SpO_2, arterial O_2 saturation; HR, heart rate; RPE, ratings of perceived exertion.

**Significantly different from sprint 1 ($P < 0.05$). #and †significantly different from NM and LA, respectively ($P < 0.05$).*

TABLE 2 | Neuromuscular parameters recorded during brief explosive maximal knee extension before (Pre-tests) and after (Post-tests) repeated sprinting in normoxia (NM; FiO_2 = 20.9%), under low (LA; FiO_2 = 16.8%) and high (HA; FiO_2 = 13.3%) normobaric hypoxia.

Variables	Pre-tests			Post-tests			ANOVA *(Partial eta-squared)*		
	NM	LA	HA	NM	LA	HA	Time	Condition	Interaction
MVC torque (Nm)	271.0 ± 45.6	269.5 ± 41.9	270.1 ± 42.3	246.5 ± 35.4*	237.6 ± 39.0*	232.3 ± 40.8*#	<0.001 *(0.77)*	0.085 *(0.19)*	0.021 *(0.28)*
Peak RTD (Nm.s)	1018 ± 122	1074 ± 208	986 ± 195	965 ± 133*	948 ± 152*	942 ± 199*	0.031 *(0.33)*	0.024 *(0.11)*	0.040 *(0.24)*
RMS_{MAX}_VL (mV)	0.604 ± 0.112	0.599 ± 0.110	0.597 ± 0.111	0.568 ± 0.107	0.524 ± 0.093	0.553 ± 0.110	<0.001 *(0.59)*	0.159 *(0.14)*	0.117 *(0.16)*
RMS_{MAX}_RF (mV)	0.449 ± 0.173	0.451 ± 0.158	0.449 ± 0.160	0.403 ± 0.162	0.398 ± 0.147	0.395 ± 0.169	<0.001 *(0.65)*	0.927 *(0.06)*	0.777 *(0.13)*
M-wave_VL (mV)	11.4 ± 4.5	12.0 ± 4.5	11.4 ± 4.6	11.5 ± 4.5	11.3 ± 4.4	11.9 ± 4.9	0.903 *(0.01)*	0.905 *(0.08)*	0.176 *(0.15)*
M-wave_RF (mV)	12.4 ± 2.0	12.6 ± 3.2	12.4 ± 2.0	13.0 ± 2.1	12.5 ± 1.4	12.8 ± 1.7	0.436 *(0.051)*	0.914 *(0.07)*	0.570 *(0.05)*
RMS_{MAX}/M-wave_VL (mV)	0.054 ± 0.027	0.052 ± 0.025	0.056 ± 0.030	0.051 ± 0.025	0.049 ± 0.029	0.048 ± 0.022	0.075 *(0.24)*	0.643 *(0.04)*	0.395 *(0.07)*
RMS_{MAX}/M-wave_RF (mV)	0.038 ± 0.018	0.039 ± 0.020	0.037 ± 0.017	0.033 ± 0.016	0.033 ± 0.013	0.031 ± 0.015	0.002 *(0.57)*	0.053 *(0.05)*	0.813 *(0.02)*

Mean ± SD (n = 13).

MVC torque, maximal voluntary contraction torque; Peak RTD, peak rate of torque development; RMS_{MAX}, M-wave and RMS_{MAX}/M-wave represent the average of root mean square, maximal M-waves, and normalized electromyogram activity of vastus lateralis (VL) and rectus femoris (RF) muscles.

**Significantly different from Pre-tests ($P < 0.05$). #Significantly different from NM ($P < 0.05$).*

of 15 5-s cycling sprints interspersed with 25-s of rest (Billaut et al., 2013). Despite not supported by statistical analysis, our novel finding was that a graded effect of hypoxia was visible for strength losses post-repeated sprinting with progressively larger values (−9, −12, and −14%) with increasing severities of acute hypoxia. This extends similar findings following maximal intermittent dynamic leg extension where KE MVC torque losses increased (yet non-significantly) with hypoxia severity (−18, −19, and −27% in FiO_2 = 21, 14, and 10%, respectively) (Christian et al., 2014). Contrastingly, performing sets of intermittent, isometric, quadriceps contractions at 60% of MVC force to task failure in normoxia, mild hypoxia, moderate hypoxia and severe hypoxia (FiO_2 = 21, 16, 13, and 10%) resulted in ∼30% declines in MVC force in all conditions, despite large differences in time-to-task failure (24.7 vs. 15.9 min in FiO_2 = 21 vs. 10%) (Goodall et al., 2010). Potentially, differences in exercise mode/duration/intensity and individuals' tolerance to hypoxic stress, in turn affecting the magnitude of reduction in convective O_2 transport, may explain aforementioned diverging results on MVC torque and further confirm that fatigue is task-dependent.

Also worth noticing, the effect of fatigue on M-wave changes during repeated sprinting is not clear-cut in literature, with reports of decreased (Perrey et al., 2010), unchanged (Girard et al., 2013), and increased (Racinais et al., 2007) amplitudes. Consequently, normalizing the raw EMG amplitude to a maximum compound action potential (M-wave) is a methodological requirement allowing control for any changes in neuromuscular junction and sarcolemma excitation, and hence, enhancing the sensitivity of EMG amplitude measurements. In this study, maximal normalized EMG activity (RMS_{MAX}/M-wave ratio) was dampened during MVCs in VL (−8%; albeit not significantly different) and RF (−14%) muscles. This suggests that the magnitude of the efferent motor outflow reaching the KE was adversely affected by the repetition of eight maximal sprints, confirming previous observations (Girard et al., 2015a; Brocherie et al., 2015b), while severity of hypoxic exposure had only minimal effect.

Rapid Muscle Force Characteristics

In addition to the post-exercise decrement in MVC torque, this study is unique as far as we are aware in reporting that the altitude severity (at least to 3600 m) did not modify the post-exercise RTD responses: the early phase RTD (30 and 50 ms) did not change, whereas the decline in peak RTD and absolute RTD values in the late phase (100 and 200 ms) of muscle contraction was similar between the normoxic and hypoxic conditions. In hot and cool conditions, a global (i.e., at all time intervals) downward-shift in the contractile RTD occurred after repeated cycling sprints (Girard et al., 2013). As it was unrelated to environmental conditions (i.e., modest hyperthermia), this was taken to reflect an overall fatigue-induced reduction in rapid muscle force characteristics. In the present study adopting a running mode, the difference between early (unchanged) and late (decreased) phases absolute RTDs may relate to the relative influence of passive stiffness in serial/lateral force transmission structures, myofiber cross-bridge kinetics and neural drive (Edman and Josephson, 2007).

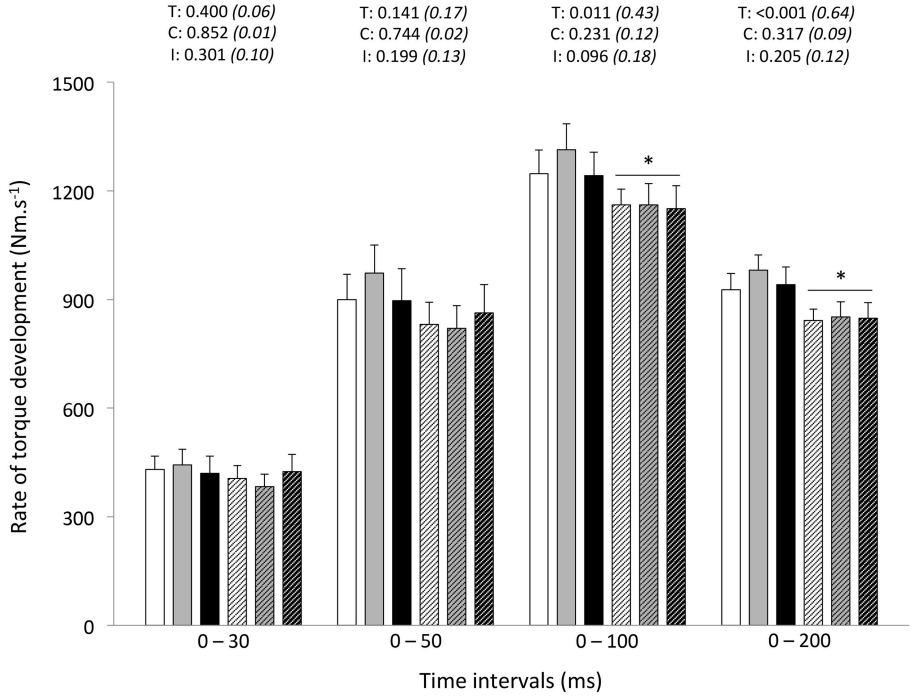

FIGURE 2 | Rate of torque development (absolute values) during explosive isometric knee extension obtained at 0–30, −50, −100, and −200 ms prior to (Pre-tests; full bars) and following (Post-tests; dashed bars) repeated sprinting in normoxia (NM; FiO_2 = 20.9%; white bars), at low (LA; FiO_2 = 16.8%; gray bars) and high (HA; FiO_2 = 13.3%; black bars) normobaric hypoxia. Values are mean ± SD (n = 13). T, C, and I respectively refer to ANOVA main effects of time, condition, and interaction between these two factors with P-value and partial eta-squared in parentheses. *Significantly different from Pre-tests, $P < 0.05$.

With late phase RTD more likely be affected by the fiber type composition (Penailillo et al., 2014), a more pronounced fatigue of faster fiber types after repeated running sprinting (Girard et al., 2015a), in turn associated with slower cross-bridge kinetics (Hamada et al., 2003), may dictate observed magnitude of post-exercise alterations in absolute RTD. Importantly, the exercise-related decline seen for the late-phase absolute RTD values occurred regardless of the environmental condition. In line with these findings, we have recently reported that heat stress does not exacerbate alterations in rapid muscle torque production capacity of KE neither after repeated cycling sprints (Girard et al., 2013) nor prolonged tennis playing (Girard et al., 2014).

In this study, when controlling for fatigue-induced reduction in maximal strength, we failed to demonstrate significant reductions in explosive strength post-exercise at any time interval. In fact, RTD is increasingly related to maximal muscle force, and reliance on muscle contractile properties decreases, as the time (mostly from 90 ms) from the onset of contraction increases (Andersen and Aagaard, 2006). The time interval in which RTD is determined influences the nature of the association between maximal muscle force and RTD and, in turn, the nature of fatigue-induced responses. By using different experimental designs (i.e., resistance training and verbal instructions during protocol), however, others have questioned the direct relationship between maximal force and RTD (Griffin and Cafarelli, 2005; Holtermann et al., 2007). Regardless, our data show the influence of maximal strength in maintaining normalized RFD values at all time intervals after repeated sprinting in differing hypoxic conditions.

Muscle Activation Rise

Whereas maximal VL muscle activation capacity (RMS_{MAX}/M-wave ratio) was impaired by sprints repetition, the ability to rapidly activate the VL muscle, as inferred from the rate of EMG amplitude rise, did not change in our study. Consistently, whole-body exercise studies, for instance following simulated team- (football: Thorlund et al., 2009; handball: Thorlund et al., 2008) and racket- (tennis: Girard et al., 2014) sport matches or a repeated cycling sprint protocol (Girard et al., 2013), show that reductions in RTDs are generally not associated with significant decreases in EMG values of quadriceps muscles. Opposite results have been reported by Morel et al. (2015) after the completion of 20 sets of 6-s isokinetic maximal KE at 240° s^{-1}, starting every 30 s. In their study, reductions of RTD were associated with a dampened activation capacity of the VL muscle in the early phase of muscle contraction, whereas participants were capable of producing similar maximal activation levels (Morel et al., 2015). Methodological differences (e.g., various time intervals, manual vs. automated methods to detect contraction onset; instructions to the participants during the contraction execution) preclude meaningful comparisons of EMG responses between studies.

In our study, the fact that RF muscle activation rise increased (at all time intervals) from pre- to post-exercise, despite

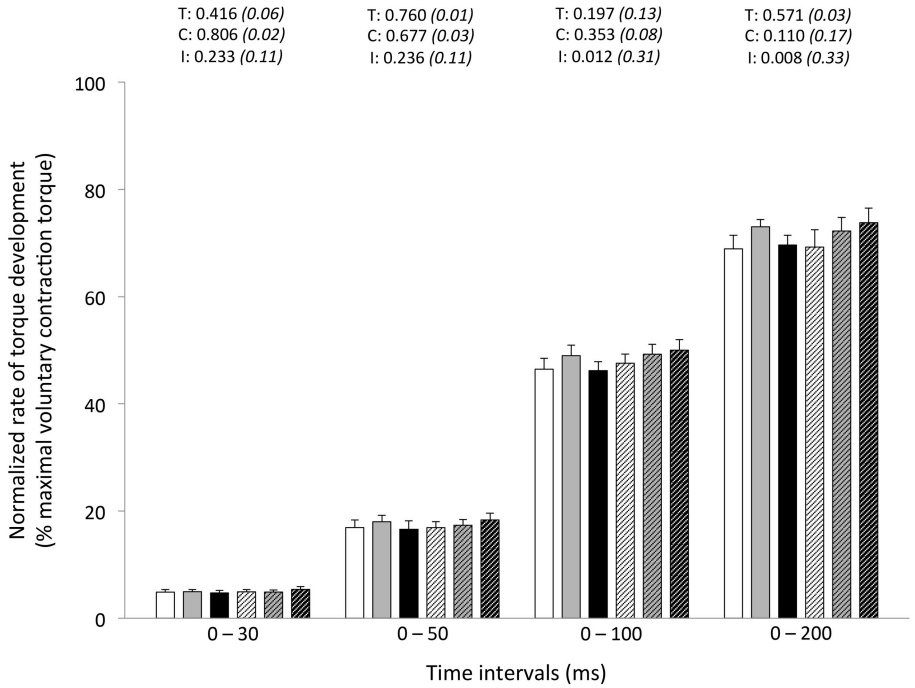

FIGURE 3 | Normalized rate of torque development (% maximal voluntary contraction torque) during explosive isometric knee extension obtained at 0–30, −50, −100, and −200 ms prior to (Pre-tests; full bars) and following (Post-tests; dashed bars) repeated sprinting in normoxia (NM; FiO_2 = 20.9%; white bars), at low (LA; FiO_2 = 16.8%; gray bars) and high (HA; FiO_2 = 13.3%; black bars) normobaric hypoxia. Values are mean ± SD (n = 13). T, C, and I respectively refer to ANOVA main effects of time, condition and interaction between these two factors with P-value and partial eta-squared in parentheses.

significant alteration of the ability to maximally activate this muscle, is an interesting observation. Considering the different activation sequences of this bi-articular muscle during sprinting (Morin et al., 2015) and the observation of reductions in RF RMS activity levels over sprints repetition (Brocherie et al., 2015b), it cannot be ruled out that this finding may partly depend on our non-specific testing position (i.e., marked hip extension while seating). Potentially, different results would occur when adopting another posture (i.e., when lying down) since knee position (RF muscle length) significantly affects quadriceps activation strategies (Krishnan et al., 2011). While this result was unexpected, larger activation in the *soleus* muscle has also been observed +24-h post-football game in hot vs. neutral environment (Girard et al., 2015c). Explosive force production depends on muscle fascicle shortening velocity and the tendon's elastic energy storage capacity, with tendon stiffness in turn affecting the time lag between muscle activation and muscle force production (i.e., electromechanical delay) (Proske and Morgan, 1987). In our study, a fixed electromechanical delay was used to determine EMG onset rise. As such, the role of tension-sensitive mechanoreceptors located in the muscle (e.g., Golgi tendon organs and muscle spindles) in influencing the tendon's stiffness, and thereby length change of the muscle fibers during explosive KE through proprioceptive feedback, probably did not change. A further result was to demonstrate that hypoxia severity had no effect on post-exercise adjustments in rapid muscle activation capacity. Potentially, longer sprints, shorter recoveries or combination of both (more intense exercise-to-rest ratios) and/or the use or severer hypoxic conditions might yield more unfavorable results with respect to RTD adjustments resulting directly to decreases in muscle activation rates if greater fatigue levels could be attained.

Additional Considerations

Importantly, a marked reduction in the intrinsic contractile capacity for explosive force production cannot be ruled out, as substantial peripheral locomotor muscle fatigue development (i.e., twitch torque decrease) usually occur as a result of repeated sprinting, independently of hypoxic severity (Billaut et al., 2013). Assessment of electrically evoked RTD can give insight into the intrinsic capacity of the muscle-tendon unit for explosive force production without the influence of voluntary control. This can be investigated by examining the response to a single or ideally high frequency contractions, such as an evoked octet (e.g., eight pulses at 300 Hz; Buckthorpe et al., 2012), to reliability evoke the maximum capacity for RTD.

In our study, all pre- and post-neuromuscular assessments were performed in normoxia with similar SpO_2 values of 96–97%. Reportedly, SpO_2 recovery response after an acute exposure to normobaric hypoxia (FiO_2 = 10%) decreasing SpO_2 to 85% is ∼2 min (Krivoshchekov et al., 2014). With hypoxic simulation applied during the repeated sprint exercise through the use of a facemask, participants were switched

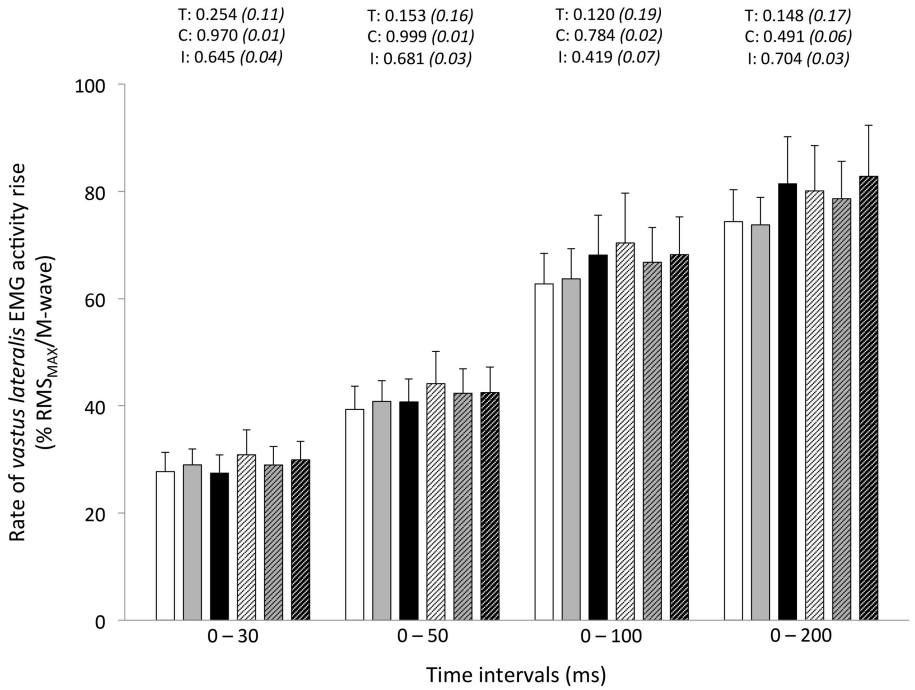

FIGURE 4 | Rate of *vastus lateralis* EMG activity rise (% RMS_{MAX}/M-wave) during explosive isometric knee extension obtained at 0–30, −50, −100, and −200 ms prior to (Pre-tests; full bars) and following (Post-tests; dashed bars) repeated sprinting in normoxia (NM; FiO_2 = 20.9%; white bars), at low (LA; FiO_2 = 16.8%; gray bars) and high (HA; FiO_2 = 13.3%; black bars) normobaric hypoxia. Values are mean ± SD (n = 13). T, C, and I respectively refer to ANOVA main effects of time, condition and interaction between these two factors with P-value and partial eta-squared in parentheses.

to normoxic breathing immediately (within seconds) after exercise cessation and seated on the chair, located near by the treadmill, for post-test assessment that started exactly 1 min after the last sprint completion. Whether this maneuver induced a faster recovery of neuromuscular function parameters, compared to situations where individuals continued breathing a hypoxic mixture (i.e., similar to exercise conditions), is unknown.

When evaluating RTD, most of the studies have used the same contraction, with a "hard and strong" instruction, to evaluate explosive and maximal voluntary strength capacities. The potential problem associated with this practice, however, is that only ballistic contractions (i.e., force production as fast as possible followed by muscle relaxation as soon the target force is reached) allow a careful evaluation of the maximal discharge rate of motor neurons (Duchateau and Baudry, 2014). Compared with a "hard-and-fast instruction," the steeper force development with a "fast" only instruction relates to a better activation of the agonist muscles at contraction onset (Sahaly et al., 2003). Although using this methodological precaution required a greater number of contractions (and potentially may have induced some recovery in neuromuscular function), we felt that it was a necessary prerequisite not to underestimate the true rate of muscle activation of our participants.

Because elite players are more accustomed to repeated-sprint activities, one can assume that, in comparison to recreational team-sport participants involved here, they may have been able to better resist fatigue. In a group of 17 healthy recreationally active individuals, those facing the greatest RTD reductions also experienced the largest fatigue rate during a Wingate cycle ergometer test and greater fatigue during an electrical stimulation protocol (Morris et al., 2010). Along the same line, only males were studied here, while females are generally less fatigable compared with men during repeated-sprint protocols (Billaut and Bishop, 2009). Whether performance level and/or gender differences exist regarding neuromuscular consequences (with special references to explosive strength), when completing repeated-sprint exercises at different altitudes, warrant further investigation.

CONCLUSION

In summary, alteration in repeated-sprint ability and post- KE MVC was greater under high altitude than in normoxia or at low altitude. In the KE, peak and late phase (>100 ms) contractile RTD decreased post-exercise to the same extent between conditions. However, contractile RTDs were not different after normalization to MVC torque, indicating that post-exercise strength losses accounted for the decrease in RTD. Additionally, we reported that repeated running sprints do not negatively influence the capacity of the central nervous

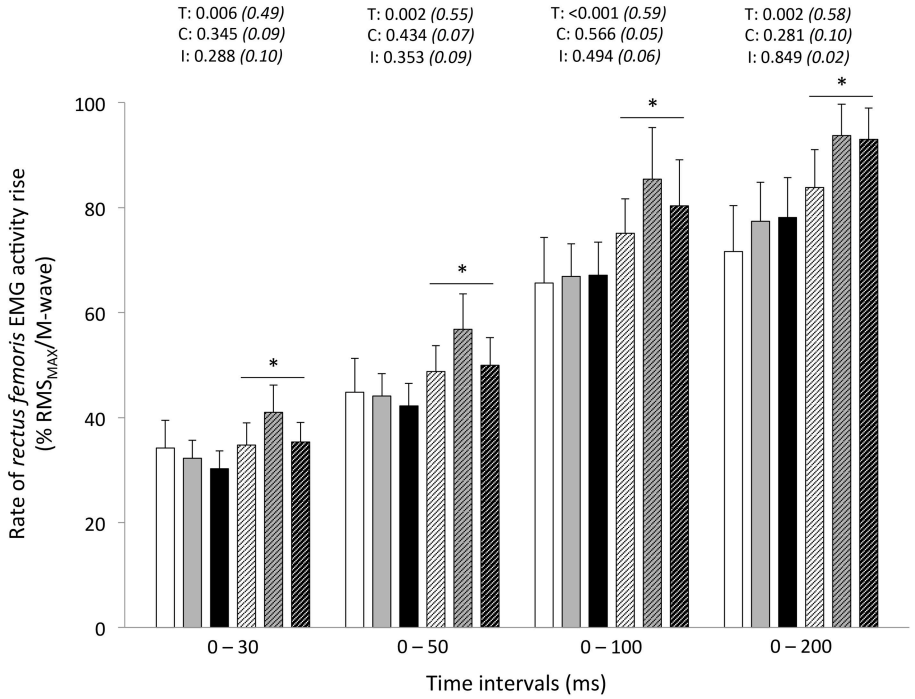

FIGURE 5 | Rate of *rectus femoris* EMG activity rise (% RMS_{MAX}/M-wave) during explosive isometric knee extension obtained at 0–30, −50, −100, and −200 ms prior to (Pre-tests; full bars) and following (Post-tests; dashed bars) repeated sprinting in normoxia (NM; FiO_2 = 20.9%; white bars), at low (LA; FiO_2 = 16.8%; gray bars) and high (HA; FiO_2 = 13.3%; black bars) normobaric hypoxia. Values are mean ± SD (n = 13). T, C, and I respectively refer to ANOVA main effects of time, condition and interaction between these two factors with P-value and partial eta-squared in parentheses. *Significantly different from Pre-tests, $P < 0.05$.

system to rapidly activate the VL (unchanged) and RF (improved) muscles during the first 200 ms, whereas maximal activation was dampened later during the contraction. Finally, normobaric hypoxia exposure had no additional influence on post-exercise alterations in rapid muscle torque production of the KE.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: OG, FB, GM. Performed experiments: OG, FB. Analyzed data: OG. Interpreted results of research: OG. Drafted manuscript and prepared tables/figures: OG. Edited, critically revised paper, and approved final version of manuscript: OG, FB, GM.

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Nitrate Intake Promotes Shift in Muscle Fiber Type Composition during Sprint Interval Training in Hypoxia

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Purpose: We investigated the effect of sprint interval training (SIT) in normoxia, vs. SIT in hypoxia alone or in conjunction with oral nitrate intake, on buffering capacity of homogenized muscle (βhm) and fiber type distribution, as well as on sprint and endurance performance.

Methods: Twenty-seven moderately-trained participants were allocated to one of three experimental groups: SIT in normoxia (20.9% F_iO_2) + placebo (N), SIT in hypoxia (15% F_iO_2) + placebo (H), or SIT in hypoxia + nitrate supplementation (HN). All participated in 5 weeks of SIT on a cycle ergometer (30-s sprints interspersed by 4.5 min recovery-intervals, 3 weekly sessions, 4–6 sprints per session). Nitrate (6.45 mmol $NaNO_3$) or placebo capsules were administered 3 h before each session. Before and after SIT participants performed an incremental VO_{2max}-test, a 30-min simulated cycling time-trial, as well as a 30-s cycling sprint test. Muscle biopsies were taken from *m. vastus lateralis*.

Results: SIT decreased the proportion of type IIx muscle fibers in all groups ($P < 0.05$). The relative number of type IIa fibers increased ($P < 0.05$) in HN ($P < 0.05$ vs. H), but not in the other groups. SIT had no significant effect on βhm. Compared with H, SIT tended to enhance 30-s sprint performance more in HN than in H ($P = 0.085$). VO_{2max} and 30-min time-trial performance increased in all groups to a similar extent.

Conclusion: SIT in hypoxia combined with nitrate supplementation increases the proportion of type IIa fibers in muscle, which may be associated with enhanced performance in short maximal exercise. Compared with normoxic training, hypoxic SIT does not alter βhm or endurance and sprinting exercise performance.

Keywords: sprint interval training, hypoxia, intermittent hypoxic training, nitrate, muscle fiber type composition, muscle buffering capacity, carnosine, citrate synthase

INTRODUCTION

Interest in intermittent hypoxic training (IHT) to boost endurance and high-intensity exercise performance in athletes is growing (Hoppeler et al., 2008; McLean et al., 2014). This might partly be explained by the commercialization of user-friendly, normobaric hypoxicators to simulate altitude within the normal lowland habitat. Well-controlled studies have provided evidence that high-intensity hypoxic endurance training can enhance muscle mitochondrial and capillary density (Geiser et al., 2001; Vogt et al., 2001; Schmutz et al., 2010; Desplanches et al., 2014), as well as stimulate other markers of mitochondrial metabolism and biogenesis (Terrados et al., 1990; Melissa et al., 1997; Green et al., 1999; Zoll et al., 2006). IHT in the form of both endurance training (Vogt et al., 2001; Zoll et al., 2006) and sprint training (Faiss et al., 2013b; Puype et al., 2013) elevated muscle phosphofructokinase (PFK) mRNA and/or activity, as well as other markers of glycolytic metabolism and pH regulation. Nonetheless, research into the effects of IHT on sea-level exercise performance is equivocal, with the current literature suggesting higher training intensities involving anaerobic energy input to be more favorable than predominantly aerobic workouts (Hoppeler et al., 2008; Faiss et al., 2013a; McLean et al., 2014). This might be explained by impaired workload in endurance training due to inhibition of oxidative energy provision, resulting in higher glycolytic energy contribution and premature fatigue development (Weyand and Lee, 1999; Calbet et al., 2003; Wehrlin and Hallén, 2006).

Attention has recently shifted toward IHT in the form of sprint training (Faiss et al., 2013b, 2015; Galvin et al., 2013; Puype et al., 2013), because maximal power (Calbet et al., 2003) and anaerobic capacity (Friedmann et al., 2007) are well-maintained in hypoxia. This may allow for more explicit systemic and muscular adaptations due to elevated hypoxic and oxidative stress in conjunction with pertinent neuromuscular and neuromechanical loading (Morales-Alamo et al., 2012; McGinnis et al., 2014). Support for such a contention comes from recent studies showing that repeated sprint training in hypoxia (RSH), characterized by several short (<30 s) sprints interspersed with incomplete recovery (exercise-to-rest ratio <1:4), increased hypoxia-inducible factor-1α (HIF-1α) mRNA (Faiss et al., 2013b), and repeated-sprint ability (RSA) performance (Faiss et al., 2013b, 2015; Galvin et al., 2013) more than identical repeated sprint training in normoxia. However, the beneficial effect of RSH on exercise performance (Faiss et al., 2015) has been debated (Montero and Lundby, 2015), and needs to be confirmed. HIF-1α is implicated in the regulation of the genes controlling the expression of proteins involved in glycolysis and pH regulation (Porporato et al., 2011). In line with this, RSH has been shown to increase gene transcription of monocarboxylate transporter 4 (MCT-4) and carbonic anhydrase III (CA3) in muscle (Faiss et al., 2013b). We have shown that 6 weeks of sprint interval training (SIT), characterized by 30-s sprints interspersed with long recovery periods of 4–5 min, increases muscle MCT-1, but not MCT-4 protein content, irrespective of whether the training was performed in normoxia or in hypoxia (Puype et al., 2013). SIT in hypoxia but not in normoxia also elevated PFK activity (Puype et al., 2013), presumably due to increased glycolytic ATP turnover to compensate for impaired aerobic energy production during the hypoxic training workouts (Weyand and Lee, 1999; Calbet et al., 2003). Given that post-exercise phosphocreatine (PCr) resynthesis is impaired under hypoxic conditions (Haseler et al., 1999; Holliss et al., 2013; Vanhatalo et al., 2014), long recovery time between sprints is required to allow substantial recovery of PCr prior to each sprint (Bogdanis et al., 1996; Parolin et al., 1999) and may assist maintenance of high power output throughout the training session.

As the contribution of glycolysis to energy provision increases, buffering capacity becomes a pivotal determinant of the capacity to maintain high muscle power outputs. Counter-intuitively, the extent of H^+ accumulation in muscle fibers during training does not seem to be the primary stimulus for the development of higher buffering capacity of homogenized muscle (βhm). Indeed, rat (Thomas et al., 2007) and human (Edge et al., 2006a) studies have shown that bicarbonate-induced myocellular alkalosis during work-matched interval training did not inhibit adaptations in βhm. Furthermore, a large acidic load (pH < 6.8) during training has been reported to reduce βhm, possibly due to cumulative transient decreases in βhm during consecutive training sessions (Bishop et al., 2008). In line with this rationale, performing SIT in hypoxic conditions might even impair βhm compared with similar training in normoxia. Alternatively, however, both "live high–train high" (Mizuno et al., 1990; Saltin et al., 1995) and "live high–train low" experiments (Gore et al., 2001) have shown that "live high" compared to "live low" enhances βhm. However, these findings have also been recently debated (Clark et al., 2004; Nordsborg et al., 2012), and the specific effect of IHT on βhm during "live low" conditions remains unclear.

Oral nitrate supplementation can enhance endurance exercise performance in hypoxia (Vanhatalo et al., 2011; Masschelein et al., 2012; Muggeridge et al., 2014), presumably by enhancing mitochondrial efficiency (Larsen et al., 2011) and/or by reducing the energy cost of muscle contraction (Bailey et al., 2010). It is well-documented that the fraction of aerobic energy provision gradually increases during intermittent sprints due to impaired re-activation of glycolysis (Parolin et al., 1999). Recent data indicate that nitrate intake increases blood flow and contractility to a greater extent in fast-glycolytic than in slow-oxidative whole muscle and muscle fibers (Hernández et al., 2012; Ferguson et al., 2013). This may explain the more explicit effects of nitrate supplementation on performance during high-intensity exercise requiring greater input of type II fibers for production of high power outputs at high contraction velocities (Vanhatalo et al., 2011; Breese et al., 2013; Bailey et al., 2015; Coggan et al., 2015). Higher muscle blood flow during recovery (Alvares et al., 2012) could conceivably facilitate the clearance of waste metabolites during intermittent maximal exercise bouts and could, amongst the other aforementioned mechanism, contribute to increased total work output during resistance training (Mosher et al., 2016). Furthermore, nitrate supplementation in hypoxia was shown to stimulate the rate of post-exercise muscle PCr resynthesis (Vanhatalo et al., 2011, 2014). Taken together, these results suggest that oral nitrate supplementation could enhance

performance during SIT in hypoxia and by this means potentiate training adaptations.

We, therefore, aimed to investigate whether SIT performed in hypoxic conditions elicited greater muscular and performance adaptations compared to similar training performed in normoxic conditions. Secondly, we aimed to investigate whether oral nitrate supplementation during training enhanced the effects of SIT in hypoxia.

MATERIALS AND METHODS

Participants

Thirty healthy men were recruited from the student population at the KU Leuven by word of mouth and via announcements on social media. To avoid confounding effects due to prior altitude acclimatization, participants who were exposed to altitudes higher than 1500 m during the 6 months prior to the study were excluded from participation. From the initial sample of 30 eligible participants, one did not complete the study due to SIT intolerance, and two withdrew from the study for reasons unrelated to the study protocol. Twenty-seven participants completed the full study protocol and were included in the final data analyses (for general characteristics see **Table 1**). Participants were recreationally active [2.7 ± 1.6 h (*SD*) exercise participation per week; i.e., soccer, basketball, cycling, running, swimming, strength training], but had not engaged in a consistent training program or any sport at a competitive level. Participants were non-smokers and did not use medication or dietary supplements in the 3 months prior to the study or during the period of the study. They were instructed to maintain their habitual physical activity level and normal diet throughout the study. Participants received a summary table of nitrate-rich foods and were instructed to avoid these foods throughout the study period. The study was approved by the KU Leuven Biomedical Ethics Committee (B322201316517) and was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent after clearing medical screening and being fully informed about the content of the experiments and the risks involved.

Study Protocol

The study involved a test before (pretest) and after (posttest) a 5-week controlled SIT program. The pretest and posttest consisted of two experimental sessions separated by a 2-day interval. Between 3 and 2 weeks prior to the start of the study, participants completed two sessions of familiarization with the experimental procedures. In the first familiarization session, participants performed a maximal incremental VO_{2max}-test on a cycle ergometer (Avantronic Cyclus II, Leipzig, Germany). The initial workload was set at 70 W and was increased by 30 W per min until volitional exhaustion. Respiratory gas exchange was measured continuously during the test (Cortex MetaLyzer II, Leipzig, Germany), and the highest oxygen uptake measured over a 30-s period was defined as the maximal oxygen uptake rate (VO_{2max}). Participants then cycled for 15 min at 50 W to recover, after which a 30-s modified Wingate test (W_{30s}) was performed. To avoid limitations of power output by co-ordination problems due to excessive cadence increase (cadence > 120 rev·min^{-1}), cadence during W_{30s} was fixed at 100 rev·min^{-1} by using the isokinetic mode setting of the cycle ergometer. In the second familiarization session, participants completed a 30-min simulated time-trial (TT_{30min}). They were instructed to keep their cadence between 80 and 100 rev·min^{-1}, and adjust the resistance to develop the highest possible mean power output (W). Following familiarization, participants were matched into triplets by VO_{2max}, mean power output during TT_{30min}, mean power output during W_{30s}, as well as body mass and height. Thereafter, the triplets were randomly assigned to one of three experimental groups. One group performed the SIT program in normoxia (F_iO_2 = 20.9%, n = 10) and received a placebo supplement (N). All other participants trained in hypoxia (F_iO_2 = 15.0%, ∼2750 m), with eight participants receiving a placebo (H) and nine participants receiving a nitrate (HN, n = 9) supplement. Participants were not blinded for the normoxic vs. hypoxic training conditions. Supplements were ingested 3 h prior to each training session so as to produce high plasma nitrite levels during the training in HN(Webb et al., 2008). Nitrate was administered in the form of capsules containing 6.45 mmol $NaNO_3$ (∼400 mg molecular NO_3^-). Placebo capsules contained an equivalent amount of sodium (6.45 mmol) in the form of NaCl. All supplements were identical in appearance, and were administered single-blinded in N and double-blinded in H and HN.

TABLE 1 | Participant characteristics.

	N	H	HN
Age (y)	23 ± 3	24 ± 2	25 ± 2
Height (cm)	180 ± 8	180 ± 6	182 ± 6
Body mass (kg)	74.0 ± 10.2	79.5 ± 12.1	78.5 ± 11.7

Data are mean ± SD and represent baseline characteristics of the participants training in normoxia (F_iO_2 = 20.9%) while receiving placebo supplementation (N, n = 10), and participants training in hypoxia (F_iO_2 = 15.0%) while receiving either placebo (H, n = 8) or nitrate supplements (HN, n = 9).

SIT Training Program

All SIT sessions were performed in the same normobaric hypoxic facility (SportingEdge, Sherfield on London, UK) set at either 20.9% F_iO_2 (N), or 15.0% F_iO_2 (∼2750 m; H and HN). The ambient O_2 fraction was checked before the start of each training session ($MaxO_2^+$A Scuba, Maxtec, Utah). Participants cycled on cycle ergometers (Avantronic Cyclus II, Leipzig, Germany) that were calibrated prior to the start of the study. Participants completed three training sessions per week, with each separated by 48-h of recovery. Each session consisted of intermittent 30-s maximal sprints, interspersed by 4.5 min active recovery intervals at 50 W. Cadence during the sprints was fixed at ∼100 rev·min^{-1} by using the isokinetic operation mode of the ergometers. The number of sprints was increased from four in weeks 1–2, to five in weeks 3–4, and six in the final week. Including 5-min warm-up and cool-down @50 Watt, the training sessions lasted

30 min in week 1, increasing to 40 min in week 5. During each session participants were given verbal encouragement to perform maximally during each sprint. To evaluate the effect of SIT on arterial oxygenation, arterial oxygen saturation (SpO_2) was monitored in the final week of the training period by pulse oximetry (Nellcor OxiMax N-600x, Mallinckrodt, St. Louis, MO) with a sensor placed 2 cm above the eyebrow.

Pretest and Posttest

All exercise testing was performed in normoxia. Participants were instructed to refrain from any strenuous physical activity for at least 48 h prior to the pretest. In order to minimize potential diet-induced variations in muscle metabolism, participants received a standardized carbohydrate-rich dinner (∼1500 kcal; 65% carbohydrate, 15% fat, 20% protein) on the evening before each experimental day. For the first session they reported to the laboratory between 12:00 a.m. and 4:00 p.m. All participants received a standardized breakfast (∼750 kcal, 70% carbohydrate, 10% fat, 20% protein) between 7:00 and 10:30 a.m. Participants completing sessions beyond 1:30 p.m. also received a standardized lunch (∼650 kcal, 70% carbohydrate, 10% fat, 20% protein), with the last meal consistently being served between 3 and 2.5 h prior to the start of the experiments. Following a 1-h rest in a comfortable chair, a percutaneous needle biopsy (100–200 mg) was taken from the middle portion of the belly of the right *m. vastus lateralis* using a 5-mm Bergström-type needle under suction. Muscle samples were dissected in two parts. One part was rapidly frozen in liquid N_2 and stored at −80°C until subsequent biochemical analyses. The other part was frozen in isopentane on liquid N_2 and stored at −80°C for later histochemical analyses. Following the biopsy, participants warmed up for 20 min at incremental workloads corresponding to 70% (10 min) and then 90% (10 min) of their average power output recorded during the TT_{30min} familiarization session. During TT_{30min} heart rate was monitored continuously (Polar, Kempele, Finland) and blood lactate concentration was measured (Lactate Pro1, Arkray, Japan) at 10-min intervals from an earlobe capillary blood sample. Participants were allowed to drink water *ad libitum* and received on-line feedback about the time remaining to completion. No verbal encouragement was given. At the end of the experimental session, participants were instructed to refrain from any strenuous physical activity, before returning to the laboratory for the second session 2 days later. For this second session they arrived between 6 a.m. and 11 a.m. after an overnight fast. Following a 20-min rest period, they performed a maximal incremental VO_{2max}-test on the cycle ergometer (Avantronic Cyclus II, Leipzig, Germany). Initial workload was set at 70 W and was increased by 30 W every 3 min until volitional exhaustion. Thereafter participants cycled for 15 min at 50 W to recover, whereupon the W_{30s} commenced. The cycle ergometer was set in the isokinetic mode with cadence fixed at 100 rev·min^{-1}. During both tests heart rate was monitored continuously (Polar, Kempele, Finland). Standardized verbal encouragement was given only during W_{30s}. Respiratory gas exchange was continuously measured (Cortex MetaLyzer II, Leipzig, Germany) during the incremental test, VO_{2max} was determined as the highest oxygen uptake rate measured over a 30-s period. Maximal power output (MPO) was calculated by summing the workload during the last full stage, plus 30 W multiplied by the fraction of the final stage completed. Capillary blood samples for lactate determination (Lactate Pro1, Arkray, Japan) were taken from the earlobe at the end of each workload during the incremental test, and power outputs corresponding to 2 and 4 mmol·L^{-1} blood lactate levels were extrapolated on the lactate-power curve. Blood lactate was also determined 1, 2, and 3 min after the W_{30s}. Room temperature (18–20°C), oxygen content (20.9%), air humidity (40%) as well as air ventilation were standardized. Pretests and the posttests were performed on the same days of the week and time of the day within each participant. The posttest commenced 3 or 4 days following the last training session to eliminate acute physiological effects due to the prior training session.

Analysis of Muscle Samples

Citrate Synthase Activity

Enzymatic activity of citrate synthase (CS) was measured by standard colorimetric method. Briefly, 5 mg of wet muscle tissue was dissolved in 400 μL of ice-cold homogenization buffer (5 mM Hepes, 1 mM EGTA, 0.1% Triton X-100, 1 mM Dithiothreitol, pH 8.7). Protein concentration was determined with a DC protein assay (Bio-Rad). After dilution to 0.5 μg·μL^{-1}, samples and standards were loaded on a 96 well plate to perform the assay in triplicate. CS catalyzes the reaction between acetyl coenzyme A and oxaloacetic acid resulting in citric acid and CoA with a thiol group (CoA-SH). Measurement of its activity is based on the binding of CoA-SH to 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB) to form 2-nitro-5-thiobenzoate (TNB). The spectrophotometric absorbance intensity of TNB was measured at 412 nm and CS activity was calculated and expressed as μmol·min^{-1}·g^{-1}. The average coefficient of variation (CV) as determined from the triplicate measures was 4.4%.

Buffering Capacity of Homogenized Muscle (βhm)

Details of the titration method for analysis of βhm have been described elsewhere (Edge et al., 2006b). Briefly, freeze-dried muscle samples (1.7–2.5 mg dm) were dissected from blood and connective tissue and homogenized on ice in a sodium fluoride containing buffer (33.3 μl 10 mM NaF per mg dm). The homogenates were warmed in a hot water bath at 37.4°C for 5 min. Basal pH measurement was performed with a glass microelectrode (MI-410, Microelectrodes, Bedford, NH, USA) connected to a pH meter (Lab 850, Schott Instruments GmbH, Mainz, Germany). The homogenates were first adjusted to pH ∼7.2 with sodium hydroxide (0.02 M NaOH). Then a serial addition of 2 μL hydrochloric acid (0.01 M HCl) was titrated until a pH of ∼6.1 was reached. After each titration, the homogenates were briefly vortexed to ensure a homogeneously mixed solution. The number of moles of H^+ per kg dry muscle required to change pH from 7.1 to 6.5 was interpolated from the fitted titration trend line and expressed as mmol H^+ per kg dm per unit pH as a unit for βhm. Each sample was measured in duplicate from which the mean was taken. The average CV as determined from the duplicate measures was 5.1%.

Muscle Carnosine Concentration

Details of muscle carnosine concentration determination by high performance liquid chromatography (HPLC) have been described elsewhere (Mora et al., 2007). Briefly, about 5 mg of dry muscle was dissected from blood and connective tissue and extracted in a buffer containing perchloric acid (0.5 M PCA) and 1 mM EDTA. After centrifuging the samples for 4 min at 13,000 rev·min^{-1} at 4°C, the supernatant was collected and neutralized with a potassium bicarbonate containing buffer (2.1 M KHCO). Samples were placed on ice for 5 min to allow CO_2 to escape, after which they were centrifuged at 5000 rev·min^{-1} for 4 min at 4°C. The supernatant was then filtered through a 0.22 μm membrane filter where after 20 μL of supernatant was injected into a Perkin-Elmer HPLC system with an Atlantis HILIC Silica column (4.6 × 150 mm, 3 μm). Mobile phase A contained 0.65 mM ammonium acetate in ultrapure water/acetonitrile (25:75 ratio) at a pH of 5.5. Mobile phase B contained 4.55 mM ammonium acetate in ultrapure water/acetonitrile (70:30 ratio) at a pH of 5.5. A linear gradient from 100% phase A to 100% phase B in 13 min at a flow rate of 1.4 mL·min^{-1} was used for separation. Separation was monitored at a wavelength of 214 nm with a UV detector. The average CV as calculated from 12 duplicate injections in the HPLC system was 1.5%.

Muscle Fiber Type Composition

Serial 7-μm-thick cryosections were cut with a cryostat at −20°C. Cryosections were blocked for 60 min in phosphate buffered saline (PBS) containing 1% BSA. Hereafter they were incubated in primary antibodies for myosin heavy chain I (MHCI) (BA-F8, Developmental Studies Hybridoma Bank) and MHCIIa (SC-71, Developmental Studies Hybridoma Bank) dissolved in PBS with 0.5% BSA for 120 min. Dilutions of primary antibodies for MHCI and MHCII were 1:50 and 1:100, respectively. After washing in PBS, cryosections were incubated in appropriate conjugated secondary antibodies (type I: Alexa 647 goat anti-mouse IgG2b, Invitrogen, diluted 1:300 in PBS with 0.5% BSA; type IIa: Alexa 350 goat anti-mouse IgG1, Invitrogen, diluted 1:300 in PBS with 0.5% BSA) for 60 min. Additionally, together with the secondary antibodies, membranes were stained using wheat germ agglutinin (WGA) Texas Red (Life Technologies). Slides were visualized by fluorescence microscopy (Nikon E1000, Nikon, Boerhavedorp, Germany). The epifluorescence signal was recorded using Cy5, DAPI, and Texas Red excitation filters for visualization of type I fibers, type IIa fibers, and cell membranes, respectively. Muscle fibers were classified as type I, type IIa, or type IIx (unstained fibers). Photos of the slides were analyzed with ImageJ software (version 1.41, National Institutes of Health, USA). Only fibers with adequate cross-sections showing no signs of distortion or folding were counted. 225 ± 27 (*SD*) fibers were analyzed per biopsy.

Statistical Analysis

Differences in baseline values between N and H and between H and HN were tested using a Student's *t*-test. Main and interaction effects were evaluated by two-way (group × time) repeated measures ANOVA (SigmaStat and SigmaPlot software, Chicago, IL, USA). We performed two separate ANOVA's to test the two *a priori* hypotheses: N was compared with H to evaluate whether SIT yielded different effects in hypoxia vs. normoxia; H was compared with HN to evaluate whether nitrate administration was able to potentiate the effects of training in hypoxia. Tukey's honestly significant difference *post hoc*-test was run whenever appropriate to identify specific effects. A probability level $P < 0.05$ was considered statistically significant. All data are expressed as mean ± standard error of the mean (*SEM*) unless otherwise stated.

RESULTS

Arterial O_2-Saturation during Training (Figure 1)

Arterial O_2-saturation (SpO_2) was continuously measured during the SIT sessions in week 5. In N, resting SpO_2-values were 98.7 ± 0.4 and 97.6 ± 0.5% at the start and at the end of the sessions (n.s.). Corresponding values in H were lower, both at the start (91.0 ± 0.8%) and at the end of the sessions (89.3 ± 1.4%; $P < 0.05$). Compared with sprint 1, post-exercise SpO_2-values were lower in the latter sprints of the session in both groups. Each sprint also reduced SpO_2 more in H (–6.0 ± 0.6%) than in N (−1.9 ± 0.2%, $P < 0.05$). SpO_2-values were not significantly different between H and HN: average SpO_2 during SIT was 85.0 ± 0.6% in H vs. 86.7 ± 0.3% in HN ($P = 0.20$).

Training Performance (Figure 2)

The number of sprints per session was increased from four in weeks 1–2, to five in weeks 3–4, and to six in week 5. Irrespective of the experimental group, mean power output in sprint 1 on average was 667 ± 31 W, decreasing to 561 ± 25 W in the final sprint of the session. There were no significant differences in power output between experimental groups at any time during training. Average total work done (kJ) per training session per week was similar between the groups from

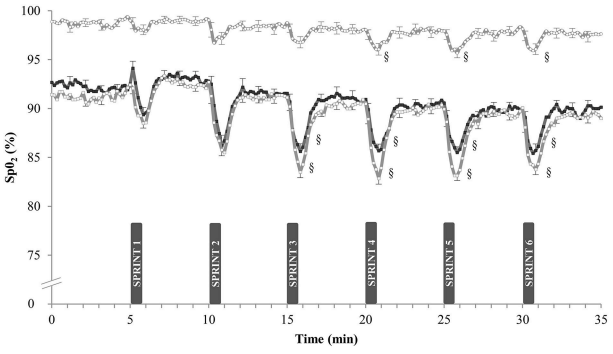

FIGURE 1 | Effect of SIT on arterial oxygen saturation. Data are mean ± SEM and represent arterial oxygen saturation (%SpO_2) during the final week of SIT. Participants performed six 30-s all-out sprints interspersed by 4 min and 30 s of active recovery on a cycle ergometer. One group trained in normoxia ($F_iO_2 = 20.9\%$) while receiving placebo (N, ○). The other groups trained in hypoxia ($F_iO_2 = 15.0\%$) while receiving either placebo (H, □) or nitrate supplements (HN, ■). §, $P < 0.05$ compared to SPRINT 1.

the start to the end of the training period (**Figure 2**). Accordingly, total work output over the 5-week training period was 1340 ± 39 kJ in N, 1311 ± 52 kJ in H, and 1261 ± 51 kJ in HN ($P > 0.50$). Blood lactate concentrations at the end of the training sessions peaked at ∼14–15 mmol·L^{-1} on average in all groups.

Exercise Performance (Table 2)

Baseline values of the incremental exercise test, TT_{30min} and W_{30s} were not significantly different between N and H ($P > 0.05$) or H and HN ($P > 0.05$). Compared with the pretest, VO_{2max} in the posttest was increased ($P < 0.05$) by ∼16% in N vs. ∼11% in both H and HN ($P < 0.05$), but there were no significant differences in VO_{2max} or change in VO_{2max} between N and H ($P = 0.26$) or between H and HN. Similarly, SIT increased ($P < 0.05$) time to exhaustion (10–11%), peak power output (8–10%), and power output corresponding to 4 mmol·L^{-1} blood lactate concentrations (5–11%) in the three experimental groups without significant differences between N and H or H and HN. Mean power output during the TT_{30min} in the pretest was, on average, ∼200 W. Training tended to increase mean power output during the TT_{30min} in N (+4%, $P = 0.062$) and significantly increased power output during the TT_{30min} in H and HN (+8%, $P < 0.05$), but there were no significant differences between N and H or H and HN. Blood lactate concentrations during the TT_{30min} were, on average, ∼6 mmol·L^{-1} at a heart rate of ∼174 b·min^{-1} in each group in both the pretest and the posttest (data not shown). Mean power output during W_{30s} in the pretest also was similar between the groups (660–670 W). SIT increased power output by ∼6% in N and H ($P < 0.05$), vs. +12% in HN ($P < 0.05$), yet differences between H and HN were not significant (HN, $P = 0.085$). Accordingly, W_{30s} produced similar peak blood lactate concentrations (∼10–12 mmol·L^{-1}) in the three experimental groups in both the pretest and the posttest.

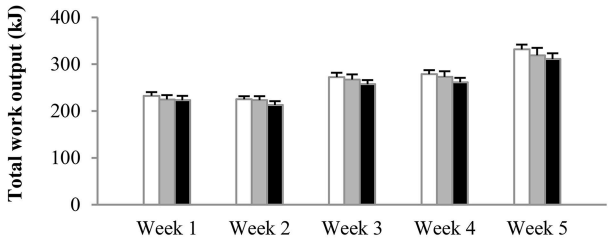

FIGURE 2 | Effect of hypoxia and nitrate intake on training workload. Data are mean ± SEM and represent average total work output (kJ) per training session per week. The number of sprints per session was increased from four in weeks 1–2, to five in weeks 3–4, and six in the final week. One group trained in normoxia ($F_iO_2 = 20.9\%$) while receiving placebo (N, open bars). The other groups trained in hypoxia ($F_iO_2 = 15.0\%$) while receiving either placebo (H, gray bars) or nitrate supplements (HN, black bars).

Muscle Fiber Type Composition (Table 3)

The relative number of type I (∼45–50%), type IIa (∼40–45%), and type IIx fibers (∼10%) in *m. vastus lateralis* in the pretest was similar between N and H ($P > 0.05$ for all fiber types) as well as between H and HN ($P > 0.05$ for all fiber types). SIT reduced the proportion of type IIx fibers in all the groups ($P < 0.05$). In the posttest the proportion of type IIa fibers was higher in HN than in H ($P < 0.05$), with training significantly increasing the proportion of IIa fibers from 45 to 56% in HN ($P < 0.05$), but not in N (main effect of time $P = 0.07$) or H ($P = 0.40$). Similar changes occurred for fiber-specific CSAs, which also were similar

TABLE 2 | Effects of training and nitrate supplementation on physiological parameters and exercise performances.

	N		H		HN	
	Pretest	**Posttest**	**Pretest**	**Posttest**	**Pretest**	**Posttest**
INCREMENTAL VO_{2max} TEST						
VO_{2max} (mL·min^{-1}·kg^{-1})	53.5 ± 2.6	62.3 ± 3.4*	54.3 ± 4.9	60.2 ± 3.5*	51.2 ± 2.2	56.9 ± 2.3*
Time to exhaustion (min)	23.1 ± 1.1	25.5 ± 1.0*	23.8 ± 1.8	26.4 ± 1.7*	22.4 ± 1.1	25.0 ± 1.2*
Peak power (W)	275 ± 12	295 ± 11*	278 ± 18	304 ± 17*	264 ± 11	290 ± 12*
Peak heart rate (beats·min^{-1})	188 ± 3	186 ± 3	188 ± 2	188 ± 3	190 ± 4	191 ± 3
Peak blood lactate (mmol·L^{-1})	11.2 ± 0.7	11.4 ± 0.4	11.0 ± 1.1	11.8 ± 1.0	12.1 ± 0.9	12.5 ± 0.6
Power output at 2 mmol·L^{-1} (W)	175 ± 10	189 ± 13#	161 ± 20	179 ± 14*	148 ± 14.1	172 ± 11*
Power output at 4 mmol·L^{-1} (W)	216 ± 10	227 ± 13*	213 ± 18	230 ± 14*	196 ± 12.0	217 ± 11*
TT_{30min}						
Mean power output (W)	203 ± 10	211 ± 12#	205 ± 16	221 ± 15*	193 ± 13	209 ± 12*
W_{30s}						
Mean power output (W)	662 ± 23	699 ± 21*	677 ± 25	719 ± 34*	663 ± 45	746 ± 41*
Peak blood lactate (mmol·L^{-1})	9.2 ± 0.7	11.1 ± 0.9*	11.5 ± 0.7	12.4 ± 0.5	9.8 ± 0.7	12.4 ± 0.4*

Data are mean ± SEM for the maximal incremental VO_{2max}-test, the 30-min simulated time-trial (TT_{30min}), and the 30-s Wingate test (W_{30s}) in the pretest and in the posttest. One group trained in normoxia ($F_iO_2 = 20.9\%$) while receiving placebo (N, n = 10). The two other groups trained in hypoxia ($F_iO_2 = 15.0\%$) and received either placebo (H, n = 8) or nitrate supplements (HN, n = 9).

**$P < 0.05$ compared to the pretest.*

#$P < 0.10$ compared to the pretest.

TABLE 3 | Effects of training and nitrate intake on muscle fiber composition.

	N		H		HN	
	Pretest	**Posttest**	**Pretest**	**Posttest**	**Pretest**	**Posttest**
RELATIVE FIBER NUMBER (%)						
Type I	49 ± 3	49 ± 3	51 ± 5	53 ± 5	45 ± 4	39 ± 2
Type IIa	42 ± 3	49 ± 3	42 ± 4	44 ± 4	45 ± 2	56 ± 2*,†
Type IIx	9 ± 2	2 ± 1*	7 ± 2	3 ± 2*	10 ± 2	5 ± 1*
RELATIVE FIBER CSA (%)						
Type I	48 ± 3	51 ± 2	48 ± 5	50 ± 5	44 ± 4	38 ± 2
Type IIa	44 ± 3	47 ± 2	45 ± 5	47 ± 4	47 ± 2	58 ± 2*
Type IIx	8 ± 2	2 ± 1*	7 ± 2	3 ± 1*	8 ± 2	4 ± 1*
FIBER CSA (μm^2)						
Type I	4769 ± 604	5214 ± 501	5269 ± 204	5843 ± 462	4998 ± 402	5106 ± 434
Type IIa	4952 ± 429	4769 ± 411	5978 ± 281	6554 ± 348	5365 ± 534	5497 ± 573
Type IIx	4507 ± 444	4302 ± 428	4929 ± 664	6078 ± 474*	3976 ± 405	4438 ± 537

Data are mean ± SEM for muscle fiber cross-sectional area (CSA) and fiber number in m. vastus lateralis in the pretest and in the posttest. One group trained in normoxia (F_iO_2 = 20.9%) while receiving placebo (N, n = 10). The other groups trained in hypoxia (F_iO_2 = 15.0%) and received either placebo (H, n = 8) or nitrate supplements (HN, n = 9).
**P < 0.05 compared to pretest.*
† P< 0.05 group × time interaction.

TABLE 4 | Effects of training and nitrate intake on biochemical measurements in muscle.

	N		H		HN	
	Pretest	**Posttest**	**Pretest**	**Posttest**	**Pretest**	**Posttest**
βhm (mmol H^+·kg dm^{-1}·pH^{-1})	136 ± 6	136 ± 6	138 ± 5	137 ± 6	125 ± 6	128 ± 6
Carnosine content (mmol·kg dm^{-1})	33.3 ± 2.3	35.3 ± 2.2	33.8 ± 2.9	38.2 ± 2.9	30.2 ± 2.2	34.0 ± 2.6
Citrate synthase activity (μmol·min^{-1}·g^{-1})	190 ± 19	292 ± 24*	231 ± 10	282 ± 16*	216 ± 17	269 ± 8.2*

Data are mean ± SEM for buffering capacity of homogenized muscle (βhm), carnosine content, and citrate synthase activity in the pretest and the posttest. One group trained in normoxia (F_iO_2 = 20.9%) while receiving placebo (N, n = 10). The other groups trained in hypoxia (F_iO_2 = 15.0%) and received either placebo (H, n = 8) or nitrate supplements (HN, n = 9).
**P < 0.05 compared to pretest.*

between N and H or H and HN in the pretest ($P > 0.05$ for all fiber types). SIT reduced the relative CSA of type IIx fibers in all the groups ($P < 0.05$). Conversely, type IIa relative CSA increased in HN (+11%, $P < 0.05$) only and was significantly greater in HN compared to H in the posttest ($P < 0.05$). SIT did not alter mean fiber CSA (μm^2) of the different fiber types, except for type IIx mean fiber CSA in H which increased from the pretest to the posttest ($P < 0.05$). However, type IIx fibers were observed in only half of the participants in H ($n = 4$), in which only few type IIx fibers were observed in the pretest [44 ± 33(*SD*)] and in the posttest [30 ± 28 (*SD*)]. Hence interpretation should be performed with caution. Mean fiber-specific CSAs were not significantly different between N and H or H and HN at any time.

Muscle Biochemistry (Table 4)

Baseline values for muscular buffering capacity, carnosine content, and maximal citrate synthase activity measured in homogenized muscle tissue were not significantly different between N and H or H and HN ($P > 0.05$). Buffering capacity of homogenized muscle was unaffected by SIT irrespective of the experimental condition. SIT on average increased muscle carnosine content by ∼13% in H and HN (main effect of time $P < 0.05$, no significant *post hoc* time effects within H ($P = 0.11$) or HN ($P = 0.13$)), but not in N (+6%, $P = 0.072$). Nonetheless, the increase in carnosine content was not significantly different between N and H ($P = 0.48$), and there were no significant differences in muscle carnosine between the groups in either the pretest or the posttest. Maximal citrate synthase activity increased by 54% in N ($P < 0.05$) and by just under half that in H (22%) and HN (25%) ($P < 0.05$), yet changes were not significantly different between N and H ($P = 0.10$).

DISCUSSION

The most striking results from the current study are that (a) 5 weeks of SIT, performed in hypoxic conditions, significantly increased the fraction of type IIa muscle fibers, but only when completed with concomitant dietary nitrate supplementation and (b) SIT performed in hypoxic conditions does not ameliorate physiological adaptations yielding enhanced aerobic or anaerobic endurance exercise performance.

We administered 400 mg of molecular nitrate ∼3 h before each SIT session in HN, which has previously been shown to significantly increase the plasma nitrite concentration (Wylie et al., 2013) and improve oxygen-efficiency of the skeletal muscle during exercise (Bailey et al., 2010; Larsen et al., 2011). We postulated that this might delay fatigue development and thereby attenuate the reduced training intensity often shown during hypoxic sprint training (Kelly et al., 2014; Thompson et al., 2015). Training workloads were, however, similar between all groups, regardless of whether the training was performed in normoxia (N) or in hypoxia, either with (HN) or without (H) nitrate supplementation. Thus, the degree of neuromechanical activation during training was similar between all experimental conditions.

Even in the absence of fiber hypertrophy, fiber type transition from IIx to IIa might be expected during short-term SIT involving 30-s sprints in healthy volunteers (for review, see Ross and Leveritt, 2001). In keeping with the published findings (Allemeier et al., 1994), 5 weeks of SIT did not induce muscle fiber hypertrophy in the current study. Consistent with this, SIT in normoxia reduced the relative type IIx fiber number, whilst the proportion of type IIa fibers tended to increase. SIT in hypoxia (H) also reduced type IIx fiber number, but did not alter the fraction of type IIa fibers. Interestingly, when nitrate supplementation was provided during SIT in hypoxic conditions (HN), relative type IIa fiber number increased from 45 to 56%.

Previous evidence indicates that nitric oxide (NO) plays a pivotal role in MHC-based muscle fiber type transition (Smith et al., 2002; Martins et al., 2012; Suwa et al., 2015). NO is suggested to increase inhibitory phosphorylation of glycogen synthase kinase (GSK)-3β in rat fast-twitch muscle, promoting nuclear factor of activated T-cell c1 (NFATc1) dephosphorylation and nuclear accumulation, resulting in a fast-to-slow fiber type transition (Martins et al., 2012). Furthermore, pharmacological inhibition of NO-synthase (NOS) activity by N^G-nitro-L-arginine-methyl-ester (L-NAME) negated overload-induced type II to I fiber type transition (Smith et al., 2002). Results from both rodent (Hernández et al., 2012; Ferguson et al., 2013) and human (Bailey et al., 2015; Coggan et al., 2015) exercise studies indicated that oral nitrate intake exerts its actions primarily in type II muscle fibers by increasing blood flow (Ferguson et al., 2013), by elevating sarcoplasmic reticulum calcium stores and the expression of calcium handling proteins such as calsequestrin 1 and the dihydropyridine receptor (Hernández et al., 2012), as well as by reducing muscle metabolic perturbation (Vanhatalo et al., 2011). Nitrate supplementation also stimulated rate of force development (Hernández et al., 2012) and power output (Bailey et al., 2015; Coggan et al., 2015) during high-intensity and high-velocity muscle contractions. Thus, while endogenous NO production during acute exposure of lowlanders to hypoxia is reduced, due to inhibition of the L-arginine–NOS pathway (Lundberg et al., 2008), exogenous nitrate provides an alternative pathway to stimulate NO production via nitrate to nitrite to NO conversion (Lundberg et al., 2008). Given that NO probably plays an important role in exercise-induced MHC-based adult fiber type transitions, it is plausible that oral nitrate intake during SIT in hypoxia served as an adequate back-up mechanism for NO-induced muscle fiber transformation to compensate for impaired NOS activity. However, in contrast to what could be expected from the current literature, i.e., stimulation of type IIx to IIa and IIa to type I fiber type transition (Smith et al., 2002; Martins et al., 2012; Suwa et al., 2015), nitrate supplementation during hypoxic SIT did not stimulate the transition of type IIa to type I muscle fibers. Follow-up studies are needed to elucidate the cellular mechanisms by which oral nitrate intake can stimulate the conversion to type IIa muscle fibers during SIT in hypoxia.

Despite a decrease in the fraction of IIx muscle fibers in all conditions and an increase in the fraction of IIa muscle fibers in HN, there was no change in βhm with training in any of the three groups. We postulated that SIT in hypoxia, due to an enhanced contribution of glycolysis to ATP production, might impair βhm compared with identical training in normoxia. Contrary to our hypothesis, however, SIT did not alter βhm, regardless of whether the training was completed in normoxia or hypoxia. Our data shows that SIT is not an adequate strategy to improve βhm, at least in healthy recreationally active volunteers during short-term training. Findings from the present and earlier studies support the opinion that high-intensity interval training (HIT) at workloads corresponding to 120–170% of the lactate threshold (Edge et al., 2006a,b) is probably more effective to raise βhm than explicit "glycolytic training" via SIT (Nevill et al., 1989; Harmer et al., 2000; Baguet et al., 2011). Adaptations of βhm during long-term SIT in elite sprinters may be different, given their substantially higher proportion of type II fibers and higher glycolytic capacity.

The physicochemical buffer capacity of muscle (∼ βhm) is composed of proteins, inorganic phosphate, bicarbonate, and the histidine-containing dipeptide carnosine. The histidine containing dipeptide content of muscle (carnosine in human muscle) contributes ∼7–8% to total βhm (Harris et al., 1990; Mannion et al., 1992; Hill et al., 2007), and elevations through training or dietary supplementation remain a plausible mechanism for increasing βhm. Accordingly, we determined the effect of SIT on muscle carnosine content and observed no significant effect in normoxic conditions. However, a main effect of time (pretest vs. posttest), though without significant *post hoc* effects, was found for the increase in muscle carnosine content following training in hypoxic conditions (H and HN), yet the training-induced changes were not significantly different between N and H. Earlier experiments in our laboratory (Puype et al., 2013) have shown that SIT in hypoxia did not increase muscle carnosine content (unpublished observations), suggesting some equivocality in the findings across our own studies. This would seem in line with the existing literature, given that SIT has previously been shown to substantially increase muscle carnosine content in one study (Suzuki et al., 2004), although no such training-effect has been shown in any other longitudinal intervention study using SIT (Baguet et al., 2011) or resistance training involving either short (∼10 s) (Kendrick et al., 2009) or longer (∼20–45 s) series of maximal muscle contractions in normoxia (Mannion et al., 1994). Future studies performing single-fiber determination of muscle carnosine are warranted for clear interpretation of

training-induced changes in fiber-specific myocellular carnosine content.

Buffering capacity of homogenized muscle, as measured in the present study, is different to muscle buffering capacity *in vivo* and reflects the altered state and chemistry of muscle after homogenization. Assuming that there would be no change in any other source of myocellular buffering, it could be estimated that a 13% increase in muscle carnosine content (i.e., 4 mmol·kg dm^{-1}) would increase homogenized muscle buffering capacity over the titration pH range of 7.1 to 6.5 by 2.21 mmol H^+·kg dm^{-1}·pH^{-1}, given that at pH 7.1 and pH 6.5 34.9 and 68.1% of the additional carnosine content is already in the protonated form, respectively (Harris et al., 1990). This represents an increase of ∼1.7% of the initial βhm in the present study and falls below the detection limit of the titration assay used. However, as we don't know what muscle buffering capacity *in vivo* is over this range, we cannot calculate the importance of this increase.

We also evaluated the effect of SIT on muscle oxidative capacity by measuring citrate synthase (CS) maximal activity. SIT substantially elevated CS activity (+25–50%), independent of whether the training was performed in normoxia or in hypoxia. These findings are in contrast with reports from single-leg studies reporting greater increases in CS activity following endurance training (30 min at 65–75% maximal work capacity) in hypoxic conditions compared to training at similar absolute workloads in normoxic conditions (Terrados et al., 1990; Melissa et al., 1997; Green et al., 1999). However, if the training workloads are matched for relative intensity, endurance training in hypoxic conditions abolishes rather than augments training-induced changes in muscle oxidative function (Bakkman et al., 2007). Training workloads during SIT in the present study were similar between normoxic and hypoxic conditions in both absolute and relative terms. In line with previous reports, SIT in normoxic conditions increased CS activity (for review see Sloth et al., 2013), but sprint training in hypoxic conditions did not augment adaptations in muscle oxidative capacity (Faiss et al., 2013a; Puype et al., 2013).

The hypothesis driving the current study was underpinned by the notion that performing SIT in hypoxic conditions, alone or in combination with oral nitrate intake, might yield specific physiological adaptations to boost exercise performance. Anaerobic glycolysis accounts for about 50% of the total ATP production during a 30-s maximal exercise bout (Putman et al., 1995). It is therefore reasonable to postulate that a higher proportion of type IIa muscle fibers, providing a higher capacity for glycolytic ATP production, should be ergogenic during a 30-s all-out exercise. As discussed above, hypoxic SIT in conjunction with oral nitrate supplementation increased relative type IIa muscle area compared with hypoxic SIT alone. Interestingly, this also tended to translate into a greater gain in power output during W_{30s} (+12% in HN vs. +6% in H, $P = 0.08$), which indicates that short-term oral nitrate supplementation in conjunction with SIT may be a valid strategy to enhance performance in "glycolytic" exercise events such as a 400-meter dash, by contributing to a beneficial fiber type shift. Future studies should seek to confirm this possibility. It is also important to emphasize that we did not study the effect of nitrate intake during SIT in normoxia, neither did we study exercise performance in hypoxia. Thus, we cannot extrapolate our findings to these conditions. Contrary to 30-s sprint performance, determinants of aerobic exercise capacity were similar between the groups. Five weeks of SIT enhanced cycling power output corresponding to 2 and 4 mmol·L^{-1} blood lactate levels, VO_{2max}, and 30-min cycling time-trial performance, independent of whether the training was performed in normoxia, or in hypoxia with or without oral nitrate supplementation. These findings indicate that short-term SIT in hypoxic compared to normoxic conditions is not an advantageous strategy for enhancing normoxic endurance exercise performance in recreationally active individuals.

In conclusion, the current experiment demonstrated that oral nitrate supplementation during short-term sprint-interval training increased the proportion of type IIa muscle fibers in muscle, which may contribute to enhanced performance in short maximal exercise events requiring a very high glycolytic rate. Compared with SIT in normoxia, SIT in hypoxia did not generate beneficial physiological adaptations yielding enhanced aerobic or anaerobic endurance exercise performance.

AUTHOR CONTRIBUTIONS

Conception and design of the study: PH and SD. All authors contributed to the collection and interpretation of the data, and reviewed and approved the final manuscript written by PH and SD.

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Similar Inflammatory Responses following Sprint Interval Training Performed in Hypoxia and Normoxia

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Sprint interval training (SIT) is an efficient intervention capable of improving aerobic capacity and exercise performance. This experiment aimed to determine differences in training adaptations and the inflammatory responses following 2 weeks of SIT (30 s maximal work, 4 min recovery; 4–7 repetitions) performed in normoxia or hypoxia. Forty-two untrained participants [(mean ± *SD*), age 21 ±1 years, body mass 72.1 ±11.4 kg, and height 173 ±10 cm] were equally and randomly assigned to one of three groups; control (CONT; no training, $n = 14$), normoxic (NORM; SIT in FiO_2: 0.21, $n = 14$), and normobaric hypoxic (HYP; SIT in FiO_2: 0.15, $n = 14$). Participants completed a $\dot{V}O_{2peak}$ test, a time to exhaustion (TTE) trial (power = 80% $\dot{V}O_{2peak}$) and had hematological [hemoglobin (Hb), haematocrit (Hct)] and inflammatory markers [interleukin-6 (IL-6), tumor necrosis factor-α (TNFα)] measured in a resting state, pre and post SIT. $\dot{V}O_{2peak}$ ($mL.kg^{-1}.min^{-1}$) improved in HYP (+11.9%) and NORM (+9.8%), but not CON (+0.9%). Similarly TTE improved in HYP (+32.2%) and NORM (+33.0%), but not CON (+3.4%) whilst the power at the anaerobic threshold (AT; $W.kg^{-1}$) also improved in HYP (+13.3%) and NORM (+8.0%), but not CON (–0.3%). AT ($mL.kg^{-1}.min^{-1}$) improved in HYP (+9.5%), but not NORM (+5%) or CON (–0.3%). No between group change occurred in 30 s sprint performance or Hb and Hct. IL-6 increased in HYP (+17.4%) and NORM (+20.1%), but not CON (+1.2%), respectively. TNF-α increased in HYP (+10.8%) NORM (+12.9%) and CON (+3.4%). SIT in HYP and NORM increased $\dot{V}O_{2peak}$, power at AT and TTE performance in untrained individuals, improvements in AT occurred only when SIT was performed in HYP. Increases in IL-6 and TNFα reflect a training induced inflammatory response to SIT; hypoxic conditions do not exacerbate this.

Keywords: high intensity training, altitude, endurance, inflammation, cytokine

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INTRODUCTION

With a training volume and energy expenditure significantly less than traditional aerobic endurance training, sprint interval training (SIT) is considered a time-efficient method of improving cardiometabolic health (Gillen et al., 2016), skeletal muscle oxidative capacity and exercise performance (Gibala et al., 2006; Burgomaster et al., 2008). SIT is characterized by repeated bouts of exercise at a supramaximal intensity, interspersed by recovery periods (Burgomaster et al., 2005). This training induces a cascade of physiological adaptations, predominantly occurring

at the muscle (metabolic adaptations), which can occur in as little as 2 weeks (Burgomaster et al., 2005; Gibala et al., 2006). Identified mechanisms facilitating improved exercise capacity via enhanced VO_2 and O_2 transport capacity include increased oxidative (Gibala et al., 2006) and glycolytic enzyme activity (Talanian et al., 2007; Daussin et al., 2008), muscle buffering capacity, glycogen content (Burgomaster et al., 2005) and increased skeletal muscle capillariation (De Smet et al., 2016; Montero and Lundby, 2016). Augmentation of exercise performance as a result of mitochondrial (Little et al., 2010) and vascular (Rakobowchuk et al., 2008) adaptations alongside improving hormonal responses (Kon et al., 2015), and insulin sensitivity (Richards et al., 2010), have reinforced SIT as a powerful training stimulus in diseased (Whyte et al., 2010), healthy untrained (Burgomaster et al., 2006; Gibala et al., 2006), and trained (Macpherson and Weston, 2015) populations.

Hypoxia has been widely observed as a potent stimuli for improving functional outcomes allied to exercise capacity (Rusko et al., 2004), with ascents ∼2500 m identified as optimal (Chapman et al., 2014) using a LHTL model for improving endurance training. A number of review articles have supported the various applications for hypoxia in trained individuals (Wilber, 2007; Millet et al., 2010, 2013). Conversely, recent discussion has proposed a limited potential for the additional benefits of adding a hypoxic stimulus to training in trained populations (Lundby et al., 2012; McLean et al., 2014). This notion may however be, too broad to suggest hypoxia is entirely ineffective. Rather the benefits of the additional hypoxic stimulus likely elicit specific adaptations allied to the protocol which has been implemented, e.g., improved repeated sprint ability after repeated sprint training in hypoxia (Faiss et al., 2013b). It has been proposed that the addition of hypoxic stress during interval training is a mechanism to further enhance performance (Faiss et al., 2013a). The application of an additional stimuli to training (i.e., hypoxia) is challenging given the necessity to maintain an optimal training stimulus, e.g., training intensity (Millet and Faiss, 2012) and optimal level of altitude (Goods et al., 2014) for beneficial adaptations. Preliminary research supports the notion that performing SIT in hypoxia may enhance the magnitude of adaptation when compared to equivalent training prescription in normoxia (Puype et al., 2013). Mechanistically, SIT in hypoxia vs. normoxia provides additive stress resulting from an increased metabolic demand to exercise and increased relative stress during recovery thus potentiating greater adaptations (Buchheit et al., 2012). Training in hypoxia still maintains the favorable time efficiencies compared to traditional continuous lower intensity training, which makes the intervention favorable for a number of applications across populations (Gibala et al., 2012). The 30 s exercise duration of each bout of SIT requires a ∼55% contribution from aerobic metabolism (Billaut and Bishop, 2009), this typically elicits greater performance detriments when training in hypoxia vs. normoxia, however prolonged recovery facilitates near complete recovery with the intention of maintaining sprint training specific stimuli (Millet and Faiss, 2012). This important balance between work:rest ratios theoretically preserves specific training stimuli associated with SIT, e.g., upregulated oxygen signaling genes and fast twitch fiber recruitment (Millet and Faiss, 2012), whilst increasing the metabolic disturbances required for adaptations to glycolytic pathways (Puype et al., 2013). Recent literature has identified that SIT in hypoxia augments adaptation during a 6 week training periods (Puype et al., 2013), however SIT in hypoxia over a 2 week training intervention may offer little additional benefit when compared to equivalent training in normoxia (Richardson and Gibson, 2015). Additional benefits of hypoxia have however also been shown in other repeated sprint training interventions of 2 (Faiss et al., 2015) to 4 weeks (Faiss et al., 2013b; Galvin et al., 2013; Kasai et al., 2015). This training modality, dose-response relationship remains to be fully determined in hypoxia, though it is known that 2 weeks is a sufficient time period to elicit adaptations in normoxia (Burgomaster et al., 2005, 2008).

Combinations of SIT and hypoxia induce significant physiological stress (Puype et al., 2013), which may impact recovery and therefore subsequent training or competition performance may be impaired (Goods et al., 2015). Interleukin-6 (IL-6) and tumor necrosis factor (TNFα) are pro-inflammatory cytokines both of which increase following equivalent training performed in hypoxia and normoxia (Svendsen et al., 2016). Plasma IL-6 increases with high intensity interval training (HIIT; Croft et al., 2009) and with increasing severity of hypoxia (Schobersberger et al., 2000; Turner et al., 2016), while TNFα appears to remain unchanged in response to passive hypoxic exposures (Turner et al., 2016). IL-6 has an important anti-inflammatory and adaptation-signaling role during the post-exercise recovery phase, with a greater increase in IL-6 post-hypoxic exercise reflective of a greater training stress (Fischer, 2006; Scheller et al., 2011). Given the failure for 2 weeks of SIT in hypoxia to elicit greater adaptations than SIT in normoxia (Richardson and Gibson, 2015) identification of the pro-inflammatory response to both interventions would facilitate greater understanding of the magnitude of additional training stimuli induced by hypoxia. Additionally, should a greater inflammatory response be identified, in the absence of improved adaptive response than equivalent training in normoxia, the use of SIT in hypoxia may be counterproductive.

The aims of this study were to investigate differences in the magnitude of training adaptations (VO_{2max}, time to exhaustion, Anaerobic Threshold) and inflammatory responses (IL-6 and TNFα), to 2 weeks of SIT performed in normoxia and hypoxia. It was hypothesized that due to the short 2-week (Richardson and Gibson, 2015), vs. longer 6 week (Puype et al., 2013), duration of the SIT, no differences in the magnitude of training adaptations would be observed. Additionally it was hypothesized that SIT performed in hypoxia will elicit greater inflammatory responses than SIT performed in normoxia due to an increased physiological stress caused by an inhibited aerobic contribution during recovery.

METHODS

Subjects

Forty-two untrained, but recreationally active individuals (27 males, 15 females) age 21 ± 1 years, body mass 72.1 ± 11.4 kg, and height 173 ± 10 cm volunteered to take part in

this experiment (**Table 1**). No differences in anthropometric or fitness measures were found between groups ($p > 0.05$). Participants were informed of the procedures to be employed in the study and associated risks, which had the approval of the University of Brighton Research Ethics Committee (ESREGC/06/14). All participants provided written, informed consent. The participants were non-smokers and had not spent time above 2000 m in the 2 months prior to the study. Participants were advised to refrain from alcohol and caffeine for 24 h prior to testing and to maintain their normal unstructured training habits (<2 hr.wk) throughout the study.

Experimental Design

The 42 participants were randomly assigned and equally split for number ($n = 14$) and sex (9 males, 5 females), to one of the three intervention groups; a normoxic (NORM) (FiO_2: 0.2093) environment, a moderate normobaric hypoxic (HYP) (FiO_2: 0.15, range 0.148–0.152; $FiCO_2$: 0.0008, range 0.0003–0.0028) environment and a control (CONT) normoxic non-training group (**Table 1**). All testing was performed in a nitrogen enriched normobaric hypoxic chamber with temperature (19°C) and humidity (40%) regulated by air conditioning (Altitude Centre, London, UK).

Familiarization of the Wingate anaerobic test (WAnT) and time to exhaustion (TTE) was performed prior to any of the experimental testing. Preliminary testing involved participants completing in sequence, a peak oxygen consumption ($\dot{V}O_{2peak}$) incremental test, a time to exhaustion cycle test (TTE) and a Wingate anaerobic test (WAnT), with 24 h separating each test. Prior to each $\dot{V}O_{2peak}$ test, venous blood was taken to measure haematocrit (Hct), hemoglobin (Hb), Interleukin- 6 (IL-6), and TNFα, see **Figure 1**. The SIT consisted of six WAnT sessions over a 2-week period with 24–48 h between each session (**Figure 1**). Each training session followed that an established SIT protocol (Burgomaster et al., 2005), consisting of an increasing number of WAnTs [four to seven 30 s "all out" efforts on a cycle ergometer interspersed with 4 min warm up/recovery (60 W)]. Throughout training heart rate [HR, bts.min^{-1} (Polar FT1, Polar Electro, Kempele, Finland)], peripheral arterial oxygen saturation [SpO_2, % (PalmSat 2500, Nonin Medical Inc., Minnesota, USA)], and rating of perceived exertion [RPE; Borg Scale 6–20 (Borg, 1982)] were measured immediately after each WAnT and every minute thereafter during recovery. Those in the CONT group maintained usual physical activity regimes for the 2 week period. Forty-eight hours after the final SIT session all participants repeated the $\dot{V}O_{2peak}$, TTE and WAnT protocols, each separated by 24 h.

TABLE 1 | Participant baseline values for anthropometric and aerobic capacity measures.

	CONT	NORM	HYP
Body Mass (Kg)	70.3 ± 13	73.3 ± 11	72.5 ± 10
Height (cm)	172 ± 10	174 ± 11	174 ± 8
Age (years)	20 ± 1	20 ± 1	20 ± 1
Hb (g.dL^{-1})	14.5 ± 1.4	14.2 ± 1.5	14.6 ± 1.8
Hct (%)	44 ± 2	45 ± 2	44 ± 2
TTE (s)	606 ± 280	589 ± 372	633 ± 330
$\dot{V}O_{2peak}$ (mL.kg^{-1}.min^{-1})	42.1 ± 9.7	42.2 ± 8.6	43.6 ± 7.9

Hb, B-Hemoglobin; Hct, Hematocrit; TTE - Time to Exhaustion.

Preliminary and Post Testing

Participants performed an incremental test to volitional exhaustion on an electromagnetically-braked cycle (Schoberer Rad Messtechnik equipped with 8 strain gauges, SRM, Germany), with the zero offset calibration procedure performed on the SRM PowerMeter prior to each test, to determine $\dot{V}O_{2peak}$. Starting at 100 W, there was a stepwise increase in power of 20 W.min^{-1}. Expired gases were obtained using a breath by breath gas analyser (Metamax 3X, Cortex, Germany). HR and RPE were taken at the end of every stage. Anaerobic threshold was computer-determined with additional visual inspection to determine the first breakpoint in ventilatory parameters.

Twenty-four hours later, participants performed a TTE at an intensity corresponding to 80% peak power output (PPO) attained during the pre-SIT assessment of $\dot{V}O_{2peak}$, on a cycle ergometer at a target cadence of 80 revs.min^{-1} (Monark, model 864, Sweden). The test was terminated at volitional exhaustion when the participants' cadence fell below 40 revs·min^{-1}; exercise duration (seconds) was then determined. Capillary blood was collected from the fingertip pre and 2 min post TTE for analysis of blood lactate (2300 Stat Plus, YSI Life Sciences, USA).

Prior to each $\dot{V}O_{2peak}$ test 10 mL of blood was collected from the ante-cubital fossa. Whole blood (∼50 μL) was divided into two heparinised capillary tubes (Hawksley & Sons Ltd., England) then centrifuged (Hematospin 1300, Hawksley & Sons Ltd., England) at 1000 rpm for 1.5 min to calculate the haematocrit using a micro haematocrit reader (Hawksley & Sons Ltd., England); the average of duplicate samples was recorded. Hemoglobin concentration (B-Hemoglobin Photometer, Hemocue, Sweden) was determined via the average of triplicate samples (B-Hemoglobin Microvettes, Hemocue, Sweden). The remaining blood was transferred into two 5 mL EDTA tubes and centrifuged at 5000 rpm for 10 min. Plasma was then extracted into microvettes and stored at –86°C. IL-6 and TNFα concentrations were analyzed using Enzyme-linked immunosorbent assays in accordance with manufacturer instructions (DuoSet ELISA Development System; R&D Systems Inc., Abingdon, UK) with corrections made for the change in plasma volume (Dill and Costill, 1974). The technical error of measurement (TEM) between duplicate samples for IL-6 was 7.1%, with a unit error value of 2.76 pg.mL^{-1} and for TNFα it was 4.1%, with a unit error value of 518.7 pg.mL^{-1}.

WAnT

Each WAnT, performed 24 h following the TTE, consisted of 30 s of "all out" maximal cycling on a friction-loaded cycle ergometer (Monark Ergomedic Peak Bike 894e, Monark Exercise AB, Sweden). The load was calculated as 7.5% body mass [0.075 kg/kg.bm^{-1}, (Bar-Or, 1987)]. The onset of each sprint was marked with a "3-2-1. GO!" countdown, and participants were instructed to cycle for ∼2 s against the ergometers inertial

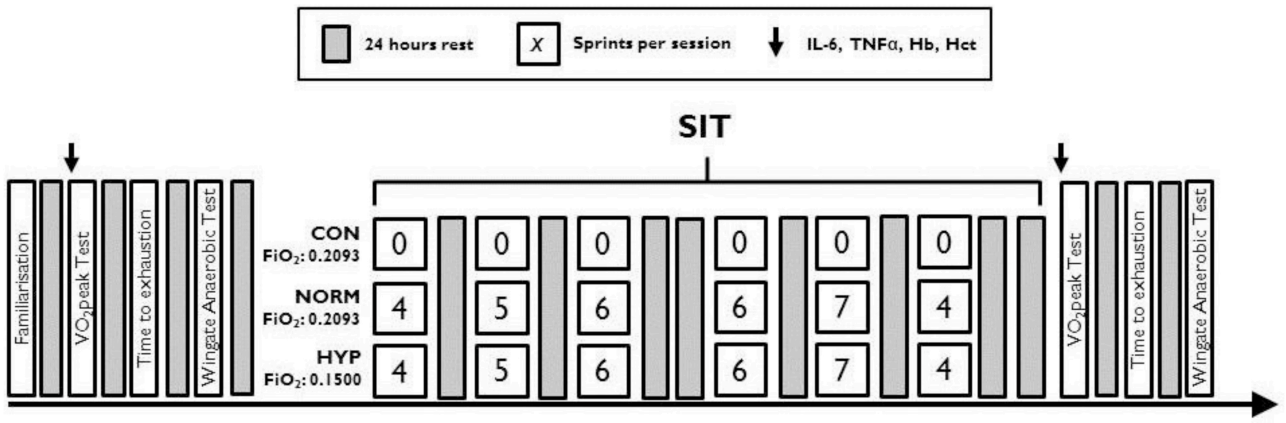

FIGURE 1 | Schematic of the testing and sprint interval training protocol for each training group.

resistance before the full load was released at 70 rpm. Participants were required to stay seated on the saddle and were verbally encouraged throughout the test. Each sprint was followed by a 4 min recovery period—participants were required actively recover (unloaded cycling at 60 revs.min^{-1}). Peak power output, average 30-s power output (total work), and fatigue index [also known as rate of power decline; ((Peak Power Output–Min Power Output)/Peak Power Output) × 100) (%)] were recorded by Monark Anaerobic Test software (ver. 3.2.7.0, Monark Exercise AB, Sweden).

Sprint Interval Training

All SIT was performed on a cycle ergometer (Monark, model 864, Sweden) against a resistance of 0.075 kg.kg^{-1} body mass, from a rolling start of 70 revs·min^{-1}. Participants were verbally encouraged throughout. The sprints were interspersed with a 4 min active recovery period of cycling at 60 W. Power measures were recorded using Monark Anaerobic Test software (Monark, Sweden) continuously throughout the sprints. The number of sprints increased from four to seven over the 2 weeks (total six sessions). Training days were interspersed with one rest day (**Figure 1**). SpO_2 and HR were monitored using a finger pulse oximeter (Nonin 2500, Nonin Medical Inc., USA) 1 min after every sprint.

Statistical Analysis

Data were tested for normality, skewness and kurtosis. Data were normally distributed unless otherwise stated. A Two Way Mixed Design ANOVA was performed separately on each of the independent variables; $\dot{V}O_{2peak}$, TTE, Peak Power, Mean Power, Fatigue index (from WAnT test), IL-6, TNFα, Hb, and Hct, to determine whether there was a significant change between the three conditions (HYP, NORM, and CON) over two time-points (pre and post). The mean sessional recovery HR and SpO_2 observed following each SIT were analyzed using a mixed 2-way ANOVA using the Greenhouse-Geisser correction to determine whether there was a significant change between the two training conditions (HYP and NORM) over the six SIT sessions. Adjusted Bonferroni comparisons were used as *post-hoc* analyses for all ANOVA. Partial eta squared was used to calculate effect sizes (np^2; small = 0.01, medium = 0.06, large = 0.13) were calculated to analyse the magnitude and trends with data. All data were reported as Mean ± *SD*. All statistical tests followed a significance level of $p < 0.05$. The statistical package used was SPSS (SPSS Inc., Chicago, USA, version 20.0).

RESULTS

Endurance Capacity

$\dot{V}O_{2peak}$ was significantly different from pre to post-training ($p = 0.001$, $np^2 = 0.44$), and between different training groups ($p = 0.002$, $np^2 = 0.28$, **Figure 2**). *Post-hoc* analysis observed increases in HYP ($p = 0.001$; +0.39 ± 0.29 L.min^{-1}; 43.6 ± 8.0 to 48.8 ± 9.2 mL.kg^{-1}.min^{-1}) and NORM ($p = 0.002$; +0.32 ± 0.38 L.min^{-1}; 42.2 ± 8.6 to 46.0 ± 7.5 mL.kg^{-1}.min^{-1}), but not for CONT ($p = 0.906$; 42.1 ± 9.7 to 42.2 ± 9.7 mL.kg^{-1}.min^{-1}). Relative power at $\dot{V}O_{2peak}$ (W.kg^{-1}) was greater pre to post ($p = 0.001$, $np^2 = 0.42$) overall and for the pre-post∗group interaction ($p = 0.004$, $np^2 = 0.25$). *Post-hoc* analysis observed increases in HYP ($p = 0.001$; 3.90 ± 0.72 to 4.20 ± 0.80 W.kg^{-1}) and NORM ($p = 0.001$; 3.54 ± 0.73 to 3.86 ± 0.86 W.kg^{-1}), but not for CONT ($p = 0.872$; 3.75 ± 0.56 to 3.76 ± 0.70 W.kg^{-1}).

The anaerobic threshold (AT) increased pre to post SIT ($p = 0.001$, $np^2 = 0.30$) overall and for the pre-post∗group interaction ($p = 0.001$, $np^2 = 0.29$). *Post-hoc* analysis only observed increases in HYP ($p = 0.001$; 22.6 ± 4.1 to 24.6 ± 4.2 mL.kg^{-1}.min^{-1}) and not NORM ($p = 0.050$; 22.9 ± 4.7 to 24.0 ± 4.8 mL.kg^{-1}.min^{-1}) or CONT ($p = 0.417$; 22.7 ± 5.6 to 22.4 ± 4.9 mL.kg^{-1}.min^{-1}). Relative power at AT was greater pre to post-training ($p = 0.001$, $np^2 = 0.313$) overall and for the pre-post∗group interaction ($p = 0.006$, $np^2 = 0.23$). *Post-hoc* analysis observed increases in both HYP ($p = 0.001$; 2.00 ± 0.36 to 2.26 ± 0.45 W.kg^{-1}) and NORM ($p = 0.017$; 1.88 ± 0.40 to 2.04 ± 0.49 W.kg^{-1}) but no change in CONT ($p = 0.925$; 1.98 ± 0.35 to 1.97 ± 0.37 W.kg^{-1}).

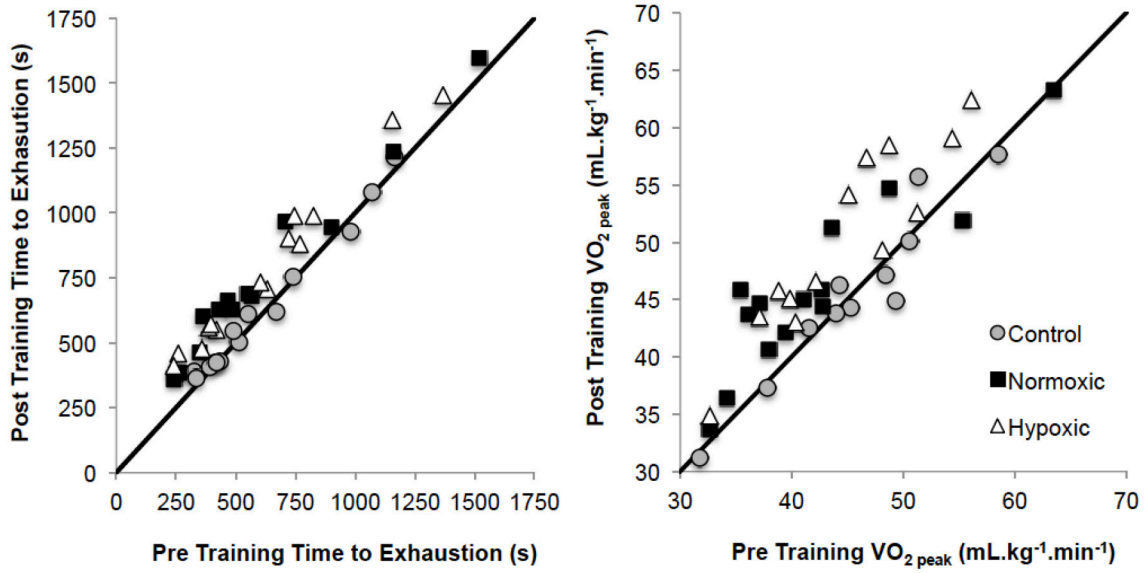

FIGURE 2 | Pre training to post training time to exhaustion and $\dot{V}O_{2peak}$ changes for the three training groups. Solid black lines demonstrates the line of equality.

TTE was significantly different from pre to post test ($p = 0.001$, $np^2 = 0.82$) overall, and for the pre-post∗group interaction ($p = 0.001$, $np^2 = 0.64$, **Figure 2**). *Post-hoc* analysis observed increases in HYP ($p = 0.001$; 633 ± 330 to 787 ± 326 s) and NORM ($p = 0.001$; 589 ± 373 to 729 ± 351 s), but not for CONT ($p = 0.212$; 607 ± 280 to 620 ± 274 s).

Power Capacity during WAnT

Peak power was not different during the WAnT from pre to post-training ($p = 0.530$, $np^2 = 0.010$) or between different training groups (CONT; 11.2 ± 3.0 to 10.9 ± 2.2 W.kg^{-1}, NORM; 11.0 ± 1.3 to 11.4 ± 1.4 W.kg^{-1}, HYP; 11.7 ± 2.4 to 11.9 ± 2.4 W.kg^{-1}) ($p = 0.052$, $np^2 = 0.141$).

Similarly, mean power was not different from pre to post-training ($p = 0.653$, $np^2 = 0.005$), or between training groups (CONT; 3.8 ± 0.8 to 3.7 ± 0.8 W.kg^{-1}, NORM; 4.0 ± 1.1 to 4.1 ± 0.9 W.kg^{-1}, HYP; 3.6 ± 1.3 to 3.8 ± 1.2 W.kg^{-1}) ($p = 0.319$, $np^2 = 0.057$).

Fatigue Index was found to be significantly reduced from pre to post-training ($p = 0.001$, $np^2 = 0.968$), although there was no significant difference between training groups CONT; 62.7 ± 10.7 to 62.9 ± 12.3%, NORM; 63.5 ± 9.2 to 61.8 ± 9.4%, HYP; 65.1 ± 12.6 to 62.3 ± 10.1%) ($p = 0.851$, $np^2 = 0.008$).

Hematological and Inflammatory Markers

Hb was significantly different from pre to post-training ($p = 0.036$, $np^2 = 0.11$). However, this increase was not different between training groups ($p = 0.082$, $np^2 = 0.12$) for CONT (14.6 ± 1.5 to 14.6 ± 1.5 g.dL^{-1}), NORM (14.3 ± 1.5 to 14.3 ± 1.3 g.dL^{-1}), and HYP (14.7 ± 1.8 to 15.0 ± 1.7 g.dL^{-1}).

Hct was not different from pre to post-training ($p = 0.701$, $np^2 = 0.00$) or between groups ($p = 0.215$, $np^2 = 0.08$) for CONT (44.0 ± 2.8 to 43.7 ± 2.4%), NORM (44.9 ± 2.1 to 45.0 ± 2.7%), and HYP (45.7 ± 2.8 to 46.0 ± 3.1%).

Blood lactate increased in all TTE tests ($p = 0.001$, $np^2 = 0.29$). Post TTE blood lactate values increased significantly with NORM ($p = 0.01$ 5.07 ± 0.77 to 5.62 ± 1.01 mmol.L^{-1}), and HYP training ($p = 0.010$; 5.24 ± 0.9 to 5.76 ± 0.82 mmol.L^{-1}), but not in CONT ($p = 0.101$; 6.42 ± 0.8 to 6.52 ± 0.95 mmol.L^{-1}).

IL-6 was significantly different from pre to post test ($p = 0.001$, $np^2 = 0.39$) and this increase was different between training groups ($p = 0.007$, $np^2 = 0.23$). *Post-hoc* analysis observed increases for HYP ($p = 0.001$; 1.7 ± 0.2 to 2.0 ± 0.2 pg.mL^{-1}) and NORM ($p = 0.003$; 1.7 ± 0.2 to 2.0 ± 0.3 pg.mL^{-1}), but not for CONT ($p = 0.836$; 1.7 ± 0.2 to 1.7 ± 0.2 pg.mL^{-1}).

TNFα was significantly different from pre to post test ($p = 0.006$, $np^2 = 0.175$), however, was not significantly different between training groups ($p = 0.151$, $np^2 = 0.09$) (NORM; 3.0 ± 0.6 to 3.4 ± 0.9 pg.mL^{-1}; HYP; 3.1 ± 0.8 to 3.3 ± 0.6 pg.mL^{-1}; CONT; 2.7 ± 0.7 to 2.8 ± 0.6 pg.mL^{-1}).

Training Markers

Recovery HR was not different between sessions ($p = 0.250$; $np^2 = 0.11$) or for the different training groups ($p = 0.420$; $np^2 = 0.04$, **Figure 3**). SpO_2 was different between sessions ($p = 0.001$; $np^2 = 0.30$) and significantly less with HYP training ($p = 0.001$; $np^2 = 0.20$). *Post-hoc* analysis of SpO_2 is presented in **Figure 3** for clarity.

DISCUSSION

The aim of this experiment was to quantify the improvements in aerobic capacity and aerobic performance following 2 weeks of SIT in normoxia, and hypoxia in comparison to a non-trained control group. In support of previous work in the field, we have

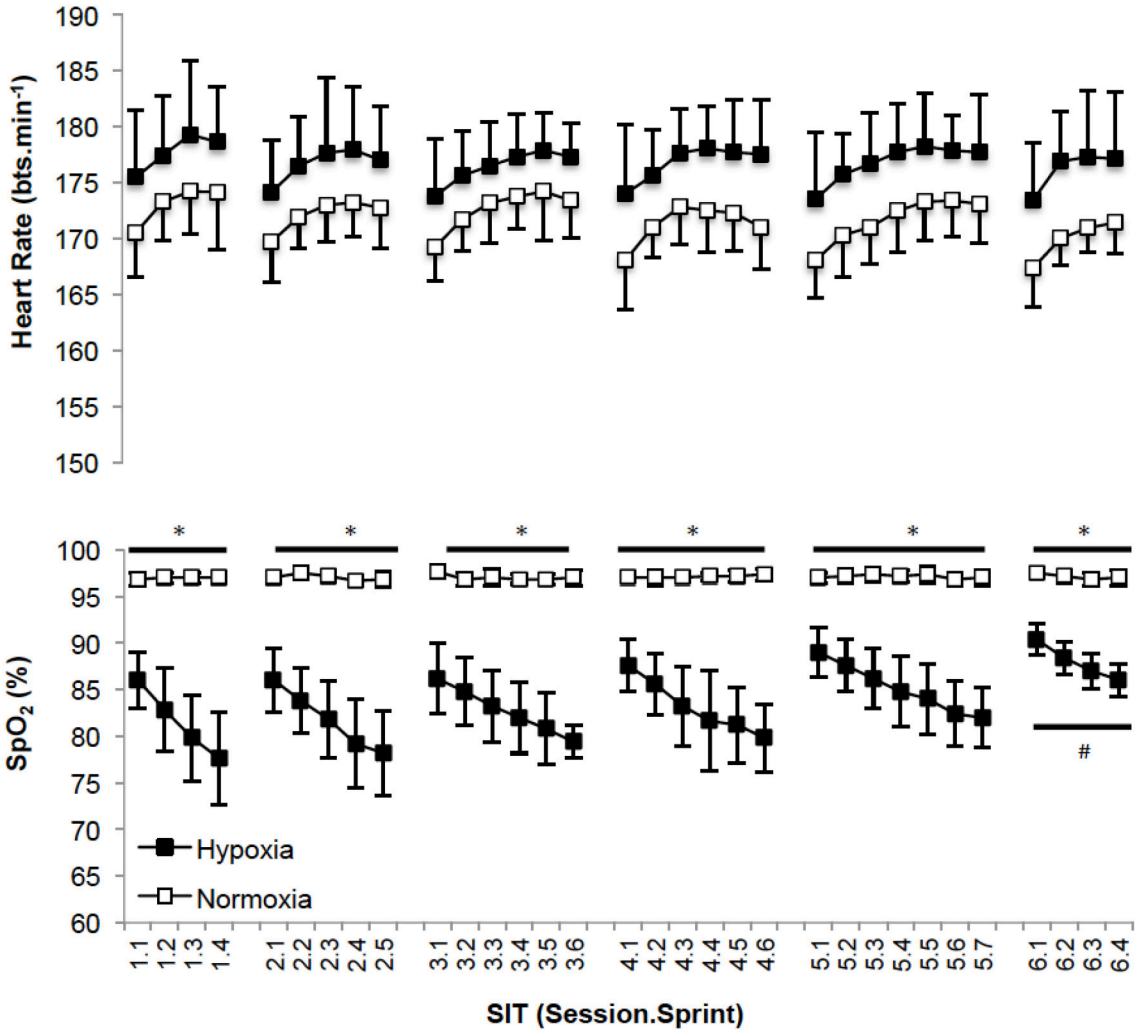

FIGURE 3 | (Mean ± *SD*) SpO_2 and heart rate values after each sprint for all training sessions. *Denotes significant difference ($p < 0.05$) between conditions within session. #Denotes significant difference ($p < 0.05$) from first, second, third, fourth and fifth sessions.

demonstrated that SIT improved $\dot{V}O_{2peak}$, power at AT and TTE to comparable magnitude associated with interval training in normoxia (Burgomaster et al., 2005; Gibala et al., 2006; Hazell et al., 2010), and hypoxia (Galvin et al., 2013; Puype et al., 2013; Gatterer et al., 2014; Brocherie et al., 2015; Richardson and Gibson, 2015; De Smet et al., 2016). In contrast to our hypothesis, an additive effect of performing SIT in hypoxia vs. normoxia was observed with regards to the AT, with no changes observed in NORM and CONT. Equality of increases in IL-6 48 h following normoxic and hypoxic SIT, also opposed our initial hypothesis. This change in concentration reflecting a training induced inflammatory response absent in controls, but with no greater, undesirable inflammatory response observed in hypoxia.

Adaptations to Aerobic Capacity and Exercise Performance

In the present study $\dot{V}O_{2peak}$, TTE, and power at the AT increased following SIT in HYP and NORM suggesting improved oxidative phosphorylation had occurred (Burgomaster et al., 2005, 2006). Interestingly, when considering AT expressed at $mL.kg^{-1}.min^{-1}$ this adaptation only occurred in HYP (+9.5%), not NORM (+5.0%). Puype et al. (2013) acknowledged that their 6-week intervention improved the power corresponding to the AT (quantified from a 4 $mmol.L^{-1}$ lactate concentration) by ∼7% (in normoxia) and 9% (in hypoxia) only following hypoxic, and not normoxic training. This adaptation was associated with an increase in muscle phosphofructokinase (PFK; hypoxia = +59%, normoxia = +17%). It was reported that VO_{2max} increased (hypoxia = +7.4%; normoxia = +5.8%) and TTE improved (hypoxia = +5.0%; normoxia = +2.9%) compared to controls, but with no difference when performing the training in hypoxia vs. normoxia (Puype et al., 2013). These observations are in line with our experiment. Our data is also supportive of an increased AT following SIT (Puype et al., 2013), and therefore increased glycolytic capacity via elevated PFK. Methodological differences in identifying the threshold,

and the three-fold protocol duration of Puype et al. (2013) provide a rationale for the contrast between significant changes in the power at the AT following training in hypoxia (Puype et al., 2013) and the absence of a further improvement in power at the AT in comparison to normoxia as observed by ourselves. The improved AT ($mL.kg^{-1}.min^{-1}$) following SIT in hypoxia in comparison to SIT in normoxia in our study may have also been acknowledged by Puype et al. (2013), unfortunately this was not quantified. SIT, or similar training, elicits known metabolic adaptations, e.g., increased oxidative (Gibala et al., 2006) and glycolytic enzyme activity (Talanian et al., 2007; Daussin et al., 2008), improved muscle buffering capacity, elevated intramuscular glycogen content (Burgomaster et al., 2005) and increased skeletal muscle capillarisation (Puype et al., 2013). These are likely induced from the performance of the sprints within the protocol, with hypoxia potentiating greater metabolic disturbances in vs. normoxia (Faiss et al., 2013b; Galvin et al., 2013; Puype et al., 2013). Even greater improvements in glycolytic function may have been identified by increased mean power and improved fatigue index during the WaNT (Puype et al., 2013), however this was not case suggesting the mode of training was not structured effectively to facilitate this. Increased lactate in response to improved TTE suggests a greater tolerance to metabolic acidosis via group III/IV afferents (Amann et al., 2015) with SIT not demonstrating improved lactate clearance as supported by evidence elsewhere (Juel et al., 2004). The absence of a difference in peak and mean power, and fatigue index during the WAnT demonstrate that SIT in normoxia and hypoxia was effective at improving aerobic metabolic pathways. Mechanisms supporting the physiological ($\dot{V}O_{2peak}$) and performance (TTE) responses in our normobaric hypoxic environment do not appear hematological given the lack of intragroup difference in Hb and Hct (**Table 2**), this is in agreement with the proposal of others (Richalet and Gore, 2008), reinforcing the metabolic adaptive pathway. The measurement of Hb and Hct would however be improved by assessing total Hb mass thus accounting for changes otherwise lost when measuring the concentration (Burge and Skinner, 1995; Schmidt and Prommer, 2005). Based upon the expected lack of hematological adaptations between groups, aforementioned modulators of oxygen utilization at the muscle are most likely improved by SIT irrespective of the FiO_2 in which it is performed. Interestingly, improvements in SpO_2 were observed within the HYP group by the 6th session (**Figure 3**). This suggests some level of acclimation to hypoxia had occurred. SIT may therefore be effective at mitigating desaturation known to occur during repeated/intermittent sprint performance in hypoxia (Bowtell et al., 2014; Turner et al., 2014). Accordingly, future work investigating training adaptations to SIT could consider the benefits of hypoxic SIT to prepare athletes, rather than untrained individuals as in the present experiment, for competition in hypoxia (Girard et al., 2013; Millet et al., 2013). Concurrent mechanistic work may also wish to consider whether our equal pre to post-intervention measures of Hb and Hct data confirms a lack of hematological adaptation i.e., HB_{mass}, in favor of improved metabolic pathways (Faiss et al., 2013b). Additionally exploration of the mechanisms by which preservation of SpO_2 occurs in response to SIT in hypoxia may also be considered. With no clear additional benefit of performing 2 weeks of SIT in hypoxia rather than normoxia (on $\dot{V}O_{2peak}$, and TTE performance lasting ∼10 min, **Table 2**), implementing this training appears largely unreasoned from a performance perspective at the present time, particularly given the challenge of facilitating training in this environment.

TABLE 2 | Change (%) in aerobic capacity, time to exhaustion (TTE), bloods and inflammatory measures in each group.

	CONT	NORM	HYP
$\dot{V}O_{2peak}$	0.9 ± 11.4	9.8 ± 9.4*	11.9 ± 6.7*
Power at $\dot{V}O_{2peak}$	−0.1 ± 5.9	8.8 ± 7.8*	7.7 ± 6.0*
AT	−0.4 ± 4.4	5.0 ± 8.2	9.5 ± 7.1*
Power at AT	−0.3 ± 12.4	8.0 ± 10.2*	13.3 ± 8.5*
TTE	3.4 ± 7.3	32.3 ± 19.0*	32.2 ± 20.7*
WAnT Peak Power	−1.7 ± 7.0	3.6 ± 3.7	1.8 ± 5.9
WAnT Mean Power	−2.4 ± 5.4	3.1 ± 9.3	4.2 ± 10.6
WAnT Fatigue Index	0.5 ± 11.4	−2.6 ± 4.7	−3.4 ± 9.5
IL-6	1.2 ± 11.8	20.1 ± 22.1*	17.4 ± 15.3*
TNFα	3.5 ± 17.5	12.9 ± 16.9*	10.8 ± 16.3*
Hb	0.1 ± 1.6	0.7 ± 4.3	2.7 ± 3.0
Hct	−0.7 ± 2.0	0.4 ± 2.7	0.7 ± 1.9

AT, Anaerobic Threshold; TTE, Time to Exhaustion; WAnT, Wingate Anaerobic Test; IL-6, Interleukin 6; TNFα, Tumor Necrosis Factor; Hb, Hemoglobin; Hct, Hematocrit.
**Denotes significant change with training.*

Inflammatory Responses to SIT in Normoxia and Hypoxia

The increase in basal IL-6 following both normoxic and hypoxic SIT (**Table 2**) reflects the intensity and duration of the activity as widely observed elsewhere (Fischer, 2006). The functional significance of an increased IL-6 is complex (Gleeson and Bishop, 2000), with increased exercise requirements (Croft et al., 2009) and increasing metabolic stress, e.g., via hypoxia in isolation, or hypoxia related increases in the relative exercise intensity (Schobersberger et al., 2000) typically eliciting larger responses. IL-6 has an important anti-inflammatory and adaptation signaling role during the post-exercise recovery phase (Svendsen et al., 2016), with a greater increase in IL-6 post-hypoxic exercise reflective of a greater training stress (Fischer, 2006; Scheller et al., 2011). A reduction of IL-6 is a known training adaptation (Fischer, 2006); the elevation of the cytokine 48 h following the final training session however indicates that recovery/adaptation was incomplete. Irrespective of the consequential effects of increased basal IL-6, the current data appeases concerns that training in hypoxia as having an impairment upon individuals when compared to equivalent normoxic training. The increase in TNF-α alongside IL-6 is similar to other data demonstrating relationships between these inflammatory biomarkers and exercise (Gleeson and Bishop, 2000). Interestingly, a similar magnitude of inflammatory response to SIT was observed for TNFα as IL-6, however this was not statistically different to controls despite the ∼12% difference between NORM and HYP, and CON. This disparity between IL-6 and TNFα may be due to the greater within group

variability observed with this inflammatory marker (∼25%) or the lack of hypoxia specific TNFα induction during passive (Turner et al., 2016) or active (Svendsen et al., 2016) exposures. Nonetheless performing SIT in hypoxia did not exacerbate the inflammatory response in comparison to normoxia, and is therefore unlikely to be detrimental to subsequent training or performance. A more precise quantification of training load within each group, and subsequently ensuring equality of load between groups, would give further confidence in the equality of inflammatory responses to SIT performed in either normoxia or hypoxia. Should absolute or relative internal/external training load be different between SIT performed in normoxia or hypoxia, then this may influence the interpretation of the inflammatory markers and suggest that hypoxia reduces or exacerbates the responses.

Similar basal inflammatory markers IL-6 and TNFα 24 h post the final SIT suggests that the increased stress of training in hypoxia is equal to that of normoxia and would not be detrimental to the individual. This equality of cytokine response is in agreement with the comparable magnitude of increases in IL-6 (hypoxia = +57%, normoxia = +56%) and lack of change in TNFα 2 h after a 75 min submaximal cycle in either condition (Svendsen et al., 2016). Accordingly the benefits of hypoxic SIT to prepare athletes for competition in hypoxia (Millet et al., 2013) can be determined at least equal to that of equivalent training in normoxia, without further compromising subsequent activities. Given the abundance of cellular and molecular pathways associated with SIT and HIIT, and hypoxia, this experiment presents a constrained overview of the responses. To optimize the application of SIT in hypoxia, future work should consider measurement of a wider spectrum of blood and muscle markers of training adaptations associated with SIT, and hypoxia both in isolation, and in combination. Additionally, further analysis of the impact of SIT in hypoxia on stress markers should be considered at a basal level (as determined within the present experiment) but also regarding the kinetics of a within session increase and the subsequent time-course to return to baseline prior to, and beyond our 48 h measurement point.

CONCLUSION

Two weeks of SIT in hypoxia improves peak oxygen uptake, time to exhaustion and power at the anaerobic threshold, to a similar magnitude as equivalent training in normoxia. Improvements in the anaerobic threshold itself were only elicited in response to SIT in hypoxia, and not normoxia, highlighting the additional benefit of training in this environment. Equality of increases in basal IL-6 and TNFα following SIT in hypoxia and normoxia suggests that hypoxia does not exacerbate inflammatory processes.

AUTHOR CONTRIBUTIONS

AR, RR, AS, and OG conceived and design the experiment. RR and AS performed the data collection. AR, RR, AS, and OG performed the statistical analysis and interpretation of data. AR, RR, AS, and OG participated in drafting the article or revising it critically for important intellectual content. AR, RR, AS, and OG approved the final manuscript.

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Hypoxic Repeat Sprint Training Improves Rugby Player's Repeated Sprint but Not Endurance Performance

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This study aims to investigate the performance changes in 19 well-trained male rugby players after repeat-sprint training (six sessions of four sets of 5 × 5 s sprints with 25 s and 5 min of active recovery between reps and sets, respectively) in either normobaric hypoxia (HYP; $n = 9$; $F_IO_2 = 14.5\%$) or normobaric normoxia (NORM; $n = 10$; $F_IO_2 = 20.9\%$). Three weeks after the intervention, 2 additional repeat-sprint training sessions in hypoxia ($F_IO_2 = 14.5\%$) was investigated in both groups to gauge the efficacy of using "top-up" sessions for previously hypoxic-trained subjects and whether a small hypoxic dose would be beneficial for the previously normoxic-trained group. Repeated sprint (8 × 20 m) and Yo-Yo Intermittent Recovery Level 1 (YYIR1) performances were tested twice at baseline (Pre 1 and Pre 2) and weekly after (Post 1–3) the initial intervention (intervention 1) and again weekly after the second "top-up" intervention (Post 4–5). After each training set, heart rate, oxygen saturation, and rate of perceived exertion were recorded. Compared to baseline (mean of Pre 1 and Pre 2), both the hypoxic and normoxic groups similarly lowered fatigue over the 8 sprints 1 week after the intervention (Post 1: −1.8 ± 1.6%, −1.5 ± 1.4%, mean change ± 90% CI in HYP and NORM groups, respectively). However, from Post 2 onwards, only the hypoxic group maintained the performance improvement compared to baseline (Post 2: −2.1 ± 1.8%, Post 3: −2.3 ± 1.7%, Post 4: −1.9 ± 1.8%, and Post 5: −1.2 ± 1.7%). Compared to the normoxic group, the hypoxic group was likely to have substantially less fatigue at Post 3–5 (−2.0 ± 2.4%, −2.2 ± 2.4%, −1.6 ± 2.4% Post 3, Post 4, Post 5, respectively). YYIR1 performances improved throughout the recovery period in both groups (13–37% compared to baseline) with unclear differences found between groups. The addition of two sessions of "top-up" training after intervention 1, had little effect on either group. Repeat-sprint training in hypoxia for six sessions increases repeat sprint ability but not YYIR1 performance in well-trained rugby players.

Keywords: normobaric hypoxia, Yo-Yo intermittent recovery test, team sports, repeated sprint ability, intermittent hypoxic training

INTRODUCTION

Rugby union is a fast-paced, field-based sport where strength, power, speed, and endurance are essential (Nicholas, 1997). Moreover, because of the game's intermittent nature, the ability to sprint repetitively is also an important fitness component for the modern rugby player and may be crucial to the outcome of the game (Austin et al., 2011a). In team sports, although different playing positions require different anthropometric and physiological characteristics, the ability to perform repeated sprints is associated with improved measures of game performance (Rampinini et al., 2007). Traditionally, repeat-sprint training involved on-feet, repeated running bouts interspersed with appropriate recovery periods (Tønnessen et al., 2011). Such training has been shown to improve oxygen utilization (Bailey et al., 2009) and increase anaerobic metabolism (Dawson et al., 1998), thereby enhancing repeat sprint ability. However, because the aerobic system is heavily involved in regenerating ATP during recovery from repeated sprints (Spencer et al., 2005), it is thought that strategies used to improve aerobic metabolism may also help to improve repeat sprint ability. As a consequence, there has been an increased interest in the ability of altitude or hypoxic training to enhance repeat sprint ability.

Increased anaerobic glycolytic activity (Svedenhag et al., 1991 #2736; Faiss et al., 2013b) and modified acid-base homeostasis (Nummela and Rusko, 2000) have both been signaled as possible mechanisms responsible for the improved repeat sprint ability after altitude/hypoxic training. A lower rate of oxygen delivery to the muscle during hypoxic training probably increases the stress on the anaerobic metabolic pathways thereby resulting in upregulation of anaerobic metabolism (Faiss et al., 2013b).

However, adding hypoxia to repeat-sprint training has not always resulted in improved repeat sprint ability at sea-level. Faiss et al. (2013b) found that two repeat-sprint training sessions per week (3 sets of 5 × 10 s all-out cycle sprinting at ∼3000 m) for 4 weeks had little effect on overall power output during a repeat sprint cycling test. Nevertheless, such training increased the number of all-out 10 s cycling sprints able to be completed prior to exhaustion in the hypoxic (3.6) compared to the normoxic (−0.4) trained groups (Faiss et al., 2013b). On the other hand, Goods et al. (2015) found 15 sessions of repeat-sprint training in hypoxia over 5 weeks (3 sets of 7 × 5 s all-out cycle sprints at ∼3000 m), had little effect compared to similar exercise in normoxia on running or cycling repeat sprint ability in Australian football players. While Galvin et al. (2013) found repeat-sprint training (12 sessions over 4 weeks of 1 set of 10 × 6 s all-out treadmill sprints at ∼3500 m) in hypoxic compared to normoxic conditions had little effect on repeat sprint ability in trained rugby players. Other researchers have reported adding hypoxia to repeat-sprint training can produce trivial (∼1.5%) (Brocherie et al., 2015a) to substantial and long-lasting beneficial effects (2.9% immediately and 2.8% 3-weeks post-training) on repeat sprint ability (Brocherie et al., 2015b). In female participants, conflicting results also exist with some researchers reporting beneficial improvements in repeat sprint ability in the hypoxic compared to the normoxic trained group (Kasai et al., 2015), while others found no such change in repeat sprint ability (Jones B. et al., 2015). For a more detailed review of the use of repeat-sprint training in hypoxia see (Faiss et al., 2013a).

Repeat-sprint training under hypoxic conditions to improve endurance performance has also resulted in conflicting results. Galvin et al. (2013) found significantly improved endurance performance (Yo-Yo intermittent recovery test level 1) after repeat-sprint training in rugby players. More recently, Jones B. et al. (2015) reported significantly faster final running velocities in female field hockey players during a 30–15 intermittent field test after repeat-sprint training under hypoxic compared to control conditions, whereas others have found little effect on 20 m shuttle run performance post hypoxic repeat-sprint training (Goods et al., 2015).

Some of the variation in response to repeat-sprint training under hypoxia between studies is probably due to the methodological differences. While most studies use a similar hypoxic training stimulus (F_IO_2 ∼14.5% equivalent to ∼3000 m), the repeat-sprint training protocols can vary considerably from repetitions of short duration high-intensity repeats (12 sessions of 1 set of 10 × 6 s; Galvin et al., 2013), to longer-duration and subsequently lower-intensity repeats (six sessions of one set of between eight and 12 × 60 s; Jones M. R. et al., 2015). Exercise prescription theory dictates that exercise training prescription should be as specific as possible to facilitate appropriate physiological adaptations (Reilly et al., 2009). Therefore, repeat-sprint training protocols should be based on work-to-rest ratio's of actual repeat sprint ability while playing the sport (adding hypoxia to this training serves to induce a larger metabolic stimulus resulting in greater adaptation). Using GPS technology (MinimaxX, Catapult Innovation, 10 Hz) Jones M. R. et al. (2015) reported the repeat sprint efforts from 33 professional rugby players over the 2012-2013 season. On average, there were ∼8 repeated high-intensity bouts (range from 5 to 11) per game, which had 3–4 efforts per bout with ∼5 s between efforts and about 6–12 min recovery between bouts (Jones M. R. et al., 2015). Using time-motion analysis of 20 professional rugby players during the 2008-2009 Super 14 international rugby competition (Austin et al., 2011b), others have reported slightly more bouts of repeat sprint efforts during the game (mean 14, range 7–17), but a similar average recovery between bouts of ∼6 min. The authors did not include the rest between efforts in each bout, so comparing the average duration of the bout (∼30 s) is difficult. To our knowledge, there are few research studies that have based their repeat-sprint training protocols on data from real matches, particularly when adding hypoxia to the training. Therefore, the aim of this study was to examine the effect of using rugby-specific repeat-sprint training with hypoxia on field-based repeat sprint and endurance ability in rugby players during pre-season training.

It has been suggested that after traditional altitude training there is a small period of attenuated performance (due to the re-establishment of sea-level training volume and intensity), followed by a longer period of improved performance (Millet et al., 2010), as adaptations to the altitude training continue to manifest over time. While the post-training performance

period requires further research, we have found that anaerobic performance in well-trained athletes started to decline back to baseline levels 9 days post-intermittent hypoxic training (Hamlin et al., 2010). Because of the natural degradation of the hypoxic adaptations over time, some practitioners have suggested using "top-up" sessions (hypoxic sessions of shorter duration than the initial hypoxic training spaced through-out the training year) in an attempt to maintain beneficial adaptations and performance (Saunders et al., 2009). The effect of a hypoxic repeat-sprint training top-up session on subsequent repeat sprint ability has not been researched to date.

Little information also exists on the minimum number of hypoxic exposures required to improve performance, or how long a possible hypoxic-induced performance enhancement remains. Therefore, a secondary aim of this study was to measure performance 3 weeks post-intervention to gain insight into the longevity of performance change, but also to investigate the effect of a 1-week top-up dose on previously hypoxic and normoxic-trained subjects.

TABLE 1 | Characteristics and baseline test 1 performance measures.

	NORM ($n = 10$)	HYP ($n = 8$)
Age (yr)	22.0 ± 4.1	20.3 ± 2.1
Body mass (kg)	88.3 ± 14.1	77.1 ± 10.2*
Height (cm)	177.9 ± 5.4	173.9 ± 4.9
Weekly training (min.wk^{-1})	248.2 ± 208.9	270.9 ± 155.8
Weekly Trimp	3463 ± 3187	3642 ± 2191
Cumulated sprint time (s)	27.4 ± 3.2	26.9 ± 3.4
Repeated sprint fatigue[1] (%)	5.5 ± 2.3	5.8 ± 3.4
Repeated sprint fatigue[2] (%)	3.5 ± 1.2	3.5 ± 1.3
Yo-Yo level 1 (m)	1100 ± 426	1200 ± 384

*Data are raw means ± SD. Weekly Trimp, weekly training impulse (training duration × intensity); Cumulated sprint time, total time for 8 sprints; Repeated sprint fatigue[1], % fatigue from sprint 1 to sprint 8 using the linear extrapolation method; Repeated sprint fatigue[2], % fatigue decrement score using the % decrement score method (100 × total sprint time/ideal sprint time) − 100; Yo-Yo level 1; distance covered in the Yo-Yo level 1 intermittent recovery test * Substantial differences.*

MATERIALS AND METHODS

Subjects

Nineteen representative and club rugby players from Canterbury, New Zealand participated in this study. Players were non-professional and played in the senior and under 21 age-group teams in the Canterbury country competition. These players typically complete two rugby-specific and 1–2 strength and conditioning sessions per week during the pre-season and two rugby-specific, one game and one recovery session per week during the regular season. The research was conducted over the whole pre-season training period which typically lasts 8–10 weeks. This study was carried out in accordance with the recommendations of the Lincoln University Human Ethics guidelines with written informed consent from all subjects. All subjects gave their written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Lincoln University Human Ethics Committee (reference 2015-46). Participant characteristics are presented in **Table 1**. All participants were healthy, free from injury, lived at sea level and had not resided at altitude within the previous 6 months. Participants were matched for baseline repeat sprint ability (i.e., cumulated time required to complete 8 × 20 m sprints), and then randomly divided into two groups: a hypoxic group (HYP, $n = 9$) and a control group (NORM, $n = 10$). Participants were asked to maintain their usual pre-season fitness and rugby training sessions throughout the study. Due to injury, one control group participant had to withdraw from the study.

Study Design

This study was single blind, placebo-controlled trial whose intervention was based on repeat sprint bouts found in real rugby matches (Austin et al., 2011b; Jones M. R. et al., 2015). Participants performed seven main trials including two baseline and five post-exposure trials. The baseline trials were performed 4–5 days apart and 1 week before beginning the first repeat-sprint training block. After 3 weeks of repeat-sprint training, three post-training trials were completed (1 week apart). A second 1-week repeat-sprint training block followed where both groups trained under hypoxia. Two further post-training trials (1-week apart) followed the "top-up" sessions (see **Figure 1**). The main trials involved field-based fitness tests including a repeat sprint ability test (8 × 20 m all-out running sprints timed to go every 20 s) and a Yo-Yo Intermittent Recovery Level 1 test (YYIR1) commonly used on rugby players (Gore, 2000). To aid in the blinding, all participants were under the impression that they would be receiving altitude training (i.e., breathing hypoxic air).

Participant Preparation

The participants were asked to arrive in a fully rested and hydrated state and to refrain from intense exercise for 24 h and caffeine for 12 h prior to each main trial. All testing was performed at the same time of day (±1 h) to minimize diurnal variation. Participants were also asked to record their dietary intake before the first baseline fitness trial to allow for replication of the diet prior to subsequent trials.

Hypoxic and Normoxic Repetitive Sprint Training

Participants were asked to complete repeat-sprint training on a Wattbike (Wattbike Pro, Nottingham, UK) at air brake resistance level 3, magnetic setting three for training sessions 1–2 and 5–8. The settings were increased for training sessions 3 and 4 (air brake level 5, magnetic setting 3) to increase overload and further challenge the participants. Prior to training, the zero was calibrated for each Wattbike according to the manufacturer's recommendations. Bike dimensions for individual athletes (saddle, handlebar heights, and positions) were initially recorded and replicated at each training session. Participants were asked to cycle in an upright, seated position. Training consisted of six sessions of repeat-sprint intervals over an initial 3 week period (two sessions per week), followed 3 weeks later by a further two top-up sessions over 1 week (**Figure 1**). For the two top-up sessions only, all participants received the normobaric

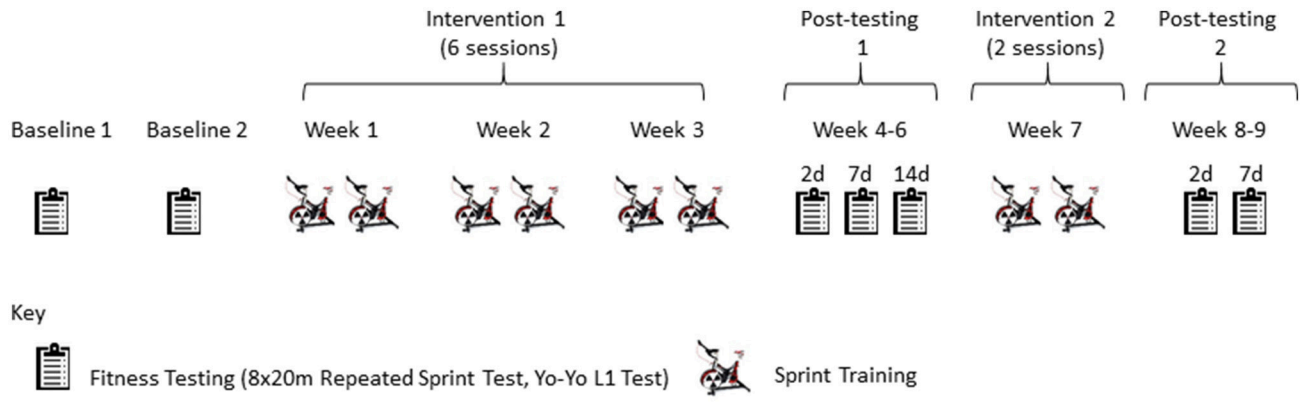

FIGURE 1 | Outline of training and testing schedule.

hypoxic gas (i.e., both the previously hypoxic and normoxic-trained groups). This protocol was to test the effectiveness of a top-up dose on previously hypoxic-trained participants and to test whether a minimum dose of two hypoxic sessions had any beneficial effect on otherwise normally trained participants. Participants maintained their normal pre-season fitness and rugby training routines. As indicated previously, the repeat-sprint interval training programme was designed around GPS data from real match play (Jones M. R. et al., 2015) and consisted of 4 sets of 5 repetitions of 5 s all-out cycling efforts interspersed with 25 s active recovery low cadence cycling (∼20–50 W) between efforts and 5 min active recovery low cadence cycling (∼20–50 W) between sets. A 5 min warm-up at ∼50 W interspersed with a 5 s sprint at the end of each minute was performed prior to the sprint training, making the total exercise time ∼35 min per session and 280 min over the total study (i.e., a total of 280 min under hypoxia). All participants were given strong verbal encouragement to maintain effort throughout the training.

During training, subjects received either a normobaric hypoxic (HYP) or a normobaric normoxic gas (NORM) via the GO_2Altitude® hypoxicator system (Biomedtech, Victoria, Australia). After calibrating the equipment at the start of each training session, the hypoxic or placebo (normoxic) gas was sent to two 100L Douglas bags connected in series. Participants breathed from the bags via a leak-free respiratory mask (Hans-Rudolph 8980, Kansas City, Missouri, USA) attached to a one-way non-rebreathing valve (Hans-Rudolph 2700). The fraction of inspired oxygen (F_IO_2) was set at 14.5% (∼3000 m) for the HYP group, and 20.9% for the NORM. We selected this hypoxic level based on previous research which suggested an F_IO_2 of between 14.8 and 16.7% (2000–3000 m) increases the physiological stress during repeat-sprint training without exacerbating the speed decline during such training (Bowtell et al., 2014; Goods et al., 2014). Participants were unable to view any oxygen or blood saturation monitors during training and we are confident of the blinding procedure as when asked at the end of intervention one, only a small fraction (i.e., 2 out of 10 controls) thought they might be receiving a higher oxygen dose or not getting hypoxia at all.

Participants recorded their daily training information along with their subjective ratings of stress, fatigue, muscle soreness, quality of sleep, and quality of training performance. Previous research by our group (Hamlin and Hellemans, 2007) and other researchers (Eston and Williams, 1988) indicates that such effort ratings can be used as reliable indicators of exercise intensity. To compare the total training load between groups, training impulse (Trimp) (Banister and Calvert, 1980) was calculated, which was expressed as a product of stress (duration of training) and strain (subjective rating of training intensity). Participants reported their subjective rating with the use of the 15-point (6–20) Borg scale (Borg, 1982).

Performance Tests

Performance testing was composed of a warm-up, a squat jump (not reported here), a repeat sprint ability test, and a Yo-Yo Intermittent Recovery Level 1 test (YYIR1) followed by a warm-down. The order of testing was standardized and participants were rested for 10–15 min between each test. The warm-up consisted of a slow jog for 5 min followed by 5–10 min of dynamic and static stretching. The repeat sprint ability test consisted of eight maximal effort running sprints timed to go every 20 s. Times (to the nearest 0.01 s) for each sprint were recorded using two sets of electronic speed-timing lights (Smartspeed, Fusion Sport Ltd, Australia). Fatigue during the 8 sprints was calculated by two methods; (i) applying a straight line to the data and estimating the predicted time in the first minus the last sprint which was log-transformed to get percent fatigue and (ii) using the percentage decrement score as described by Glaister et al. (2008) (Fatigue = (100 × (total sprint time/ideal sprint time)) − 100). Finally, participants completed a 20-m shuttle run test (Yo-Yo Intermittent Recovery test Level 1, BangsboSport, Denmark, YYIR1). Testing was completed at the same time of day in a covered stadium on slip-free flooring under similar climatic conditions.

Physiological Measures

During the training sessions, heart rate was recorded (FT1; Polar, Kemple, Finland) and arterial oxygen saturation (SpO_2,

Sport-Stat; Nonin Medical, Minneapolis, Minnesota, USA) was monitored manually by the researchers, which was unable to be viewed by the participants. Participant's perceived exertion during sprint training (RPE) was recorded at the end of each set with the Borg scale (6–20).

Statistical Analysis

Changes in the mean of the variables and standard deviations representing the between-and within-subject variability were estimated using a mixed modeling procedure (Proc Mixed) in the Statistical Analysis System (Version 9.3, SAS Institute, Cary North Carolina, USA). We analyzed the natural logarithm of each measure to reduce any effects in non-uniformity of error and to obtain changes in measures and errors as percentages (Hopkins et al., 2009). The fixed effects were test time (the average of baseline 1 and 2, post 1, post 2 etc.), group (HYP, NORM) and their interaction. The random effects were subject and residual variance. Chances that the true effects were substantial were estimated with a spreadsheet (Hopkins, 2006), when a value for the smallest worthwhile effect was entered. We used a value of 1% for performance measures (Paton et al., 2001). For non-performance measures, we chose 0.20 standardized units (change in mean divided by the between-subject *SD* at baseline) as the smallest worthwhile change (Cohen, 1988). To make inferences about the true (population) values of the effect of hypoxia on performance, *P*-values, and statistical significance were not used. Instead, uncertainties in the estimate of changes were presented as 90% confidence intervals and as likelihoods that the true value of the effect was increased, decreased or trivial. The descriptors: increased, trivial or decreased were used to describe the direction of the change. Where the confidence interval spanned all three possibilities (increased, trivial and decreased), the result was deemed unclear. In all other cases, such as no overlap, or an overlap between two possibilities (trivial and increased, or trivial and decreased) a clear result was achieved. Finally, the magnitude or probability of the change was assessed using a qualitative scale defined as: <0.5%: almost certainly not; <5%: very unlikely; <25%: unlikely/probably not; 25–75%: possibly, possibly not; >75%: likely, probably; >95%: very likely; and >99.5%: almost certainly.

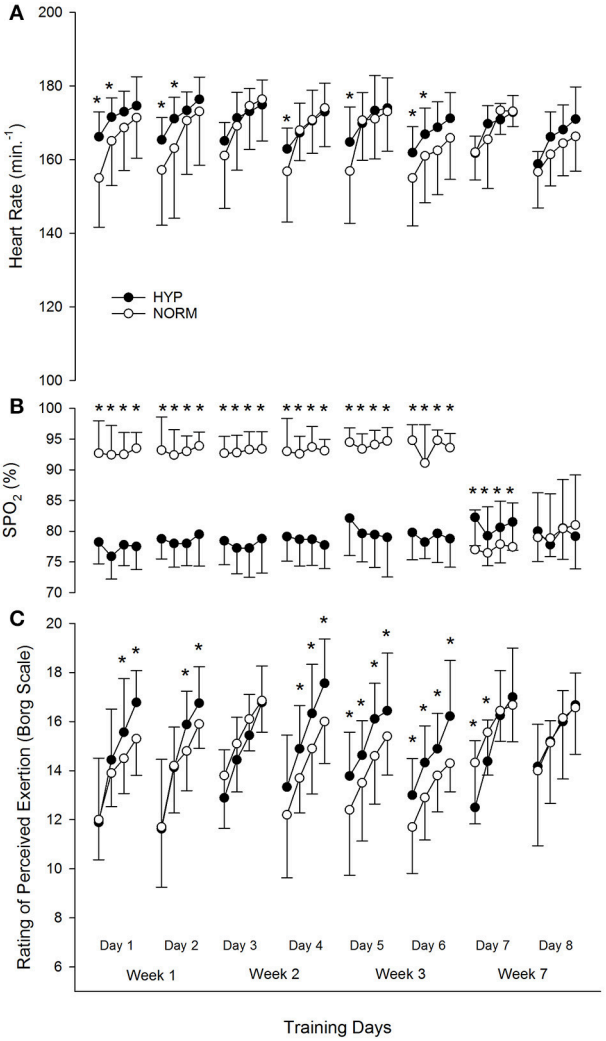

FIGURE 2 | Physiological and perceived exertion data. *Substantially different between groups at each time point. **(A)** Heart rate at the end of each set; **(B)** Arterial oxygen saturation at the end of each set; **(C)** Rating of perceived exertion (Borg 6–20 scale) at the end of each set.

RESULTS

Training Variables

We found no substantial difference in the training volume between groups measured as either training duration or training impulse (Trimp) per week (**Table 1**). The NORM group's mean SpO_2 at the end of each sprint training set remained between 92 and 95% for the first 6 training days, whereas the HYP group's SpO_2 was substantially lower at 77–82%. However, during the last 2 training days where all individuals (HYP and NORM groups) received the hypoxic gas, SpO_2 levels were lower in the NORM group compared to the HYP group initially (training day 7) but was similar in both groups by training day 8 (**Figure 2B**). Relative to the NORM group, the HYP group's heart rate at the end of each sprint training set was consistently elevated, particularly in the first 2 sets throughout the training period, apart from the last 2 training days when both groups received the hypoxic gas during training where heart rates were similar. Ratings of perceived exertion tended to be higher after the last set of each training day. Overall, perceived exertion tended to be higher in participants undertaking repeat sprint training under hypoxic compared to normoxic conditions (**Figure 2C**). Similar to SpO_2 and heart rate, the first day of hypoxic training for the NORM group (training day 7) increased their perceived exertion during training, compared to the HYP group. The average peak power measured in Watts produced during the sprint training (mean of the 20 repeat sprints for each day) was substantially higher in the NORM compared to the HYP group on days 4–6 (**Table 2**), however this difference disappeared once body weight was accounted for (i.e., W kg^{-1}). The average peak power was maintained throughout the 8

TABLE 2 | Peak power output for each training day.

		Intervention 1						Intervention 2	
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Peak power (W)	HYP	822 ± 217	851 ± 194	821 ± 182	824 ± 181*	811 ± 209*	838 ± 196*	829 ± 220	906 ± 112
	NORM	985 ± 146	996 ± 183	953 ± 195	1012 ± 203	996 ± 203	1029 ± 207	911 ± 237	979 ± 163
Peak power (W kg^{-1})	HYP	10.2 ± 3.0	10.3 ± 3.0	10.2 ± 2.7	10.2 ± 2.5	9.98 ± 2.8	10.4 ± 3.1	10.4 ± 3.0	10.8 ± 2.8
	NORM	11.4 ± 2.3	11.4 ± 2.5	11.3 ± 2.1	11.6 ± 2.5	11.4 ± 2.6	11.8 ± 2.4	10.5 ± 2.9	11.0 ± 2.1

*Data are mean ± SD. HYP, hypoxic group; NORM, normoxic group; Peak power, the average peak power from the WattBike produced during the 20 all out 5-s sprints on each training day. *Substantially different between groups.*

TABLE 3 | Mean changes in performance tests post hypoxic and placebo exposures and the chances that the true differences in changes between groups is substantial.

		Change in mean (%)			Chances that the differences are substantial	
		Within-group, from baseline		Between-group		
Variable	Post-test	HYP ± 90% CL	NORM ± 90% CL	Difference ± 90% CL	%	Qualitative inference
Repeated sprint fatigue[1]	1	−1.8 ± 1.6*	−1.5 ± 1.4*	0.3 ± 2.2	30	Unclear
	2	−2.1 ± 1.8*	−0.9 ± 1.5	1.2 ± 2.4	55	Unclear
	3	−2.3 ± 1.7*	−0.3 ± 1.7	2.0 ± 2.4^	75	Possibly beneficial
	4	−1.9 ± 1.8*	0.4 ± 1.6	2.2 ± 2.4^	81	Likely beneficial
	5	−1.2 ± 1.6*	0.5 ± 1.7	1.6 ± 2.4^	67	Possibly beneficial
Repeated sprint fatigue[2]	1	−0.6 ± 0.9*	−0.1 ± 0.8	0.5 ± 1.2	27	Unclear
	2	−0.9 ± 0.9*	0.1 ± 0.8	1.0 ± 1.3^	50	Possibly beneficial
	3	−1.2 ± 0.9*	−0.3 ± 0.9	0.9 ± 1.4^	46	Possibly beneficial
	4	−0.6 ± 0.8*	0.4 ± 0.9	1.0 ± 1.3^	53	Possibly beneficial
	5	−0.1 ± 0.9	1.1 ± 0.9*	1.2 ± 1.2^	61	Possibly beneficial
Yo-Yo L1	1	15 ± 30	20 ± 26*	6 ± 39	58	Unclear
	2	23 ± 31	26 ± 26*	3 ± 38	53	Unclear
	3	26 ± 31*	13 ± 28	−13 ± 42	68	Unclear
	4	37 ± 31*	26 ± 28*	−11 ± 42	65	Unclear
	5	33 ± 29*	25 ± 31*	−9 ± 42	62	Unclear

Substantially different from mean of baseline tests (see **Table 1); ^Substantially difference between groups. Repeated sprint fatigue[1], % fatigue from sprint 1 to sprint 8 using the linear extrapolation method; Repeated sprint fatigue[2], % fatigue decrement score using the % decrement score method (100 × (total sprint time/ideal sprint time) − 100); Yo-Yo L1 is % change in meters covered in the Yo-Yo intermittent recovery test Level 1.*

training days despite the increased resistance on training days 3 and 4.

Performance

Initially groups were matched for repeat sprint ability using the cumulated sprint time from test 1 (HYP 27.5 ± 3.9, NORM 27.4 ± 3.2) which was altered slightly due to the withdrawal of one participant's data from the hypoxic group (HYP 26.9 ± 3.4). Fatigue during the repeat sprint ability test was similar between groups at the two baseline tests when measured using either the linear extrapolation method (Baseline 1 test, HYP 5.8 ± 3.4%, NORM 5.5 ± 2.3% and Baseline 2 test, HYP 6.1 ± 2.6%, NORM 5.9 ± 1.8%, mean ± *SD*), or the percent decrement method (Baseline 1 test, HYP 3.5 ± 1.3%, NORM 3.5 ± 1.2% and Baseline 2 test, HYP 4.1 ± 1.1%, NORM 3.9 ± 1.4%). Using the linear extrapolation approach to measure fatigue, compared to the mean of the two baseline tests the HYP participants had substantially less fatigue during the repeated sprint tests on all post-test occasions (**Table 3**), however, this was only found in the NORM participants at post-test 1. Compared to the NORM participants, a beneficial effect of adding hypoxia to the repeat sprint training was indicated from post-test 3 onwards. Similar results were found using the percent decrement method to calculate fatigue (**Table 3**). Repeat sprint times for the 2 groups for each test day are presented in **Figure 3**. Test 1 had the lowest times for each sprint in the NORM group, whereas the lowest sprint times were witnessed on test day 5 and 6 (i.e., post-test intervention 3 and 4) for the HYP group. Cumulative times for the 8 sprints were similar between groups for the baseline and early post-intervention tests (post 1 and 2), however

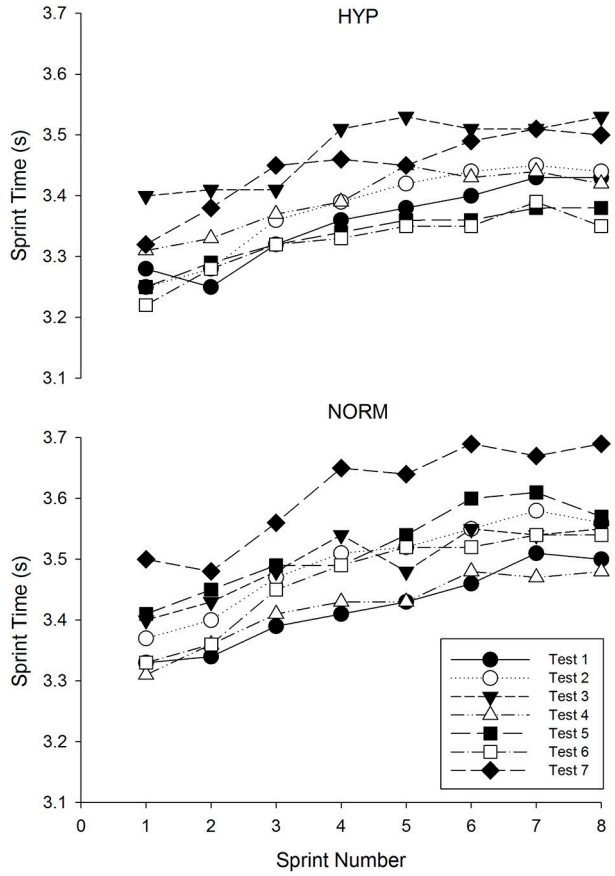

FIGURE 3 | Raw sprint times for the hypoxic (HYP) and normoxic (NORM) groups over the 7 testing periods.

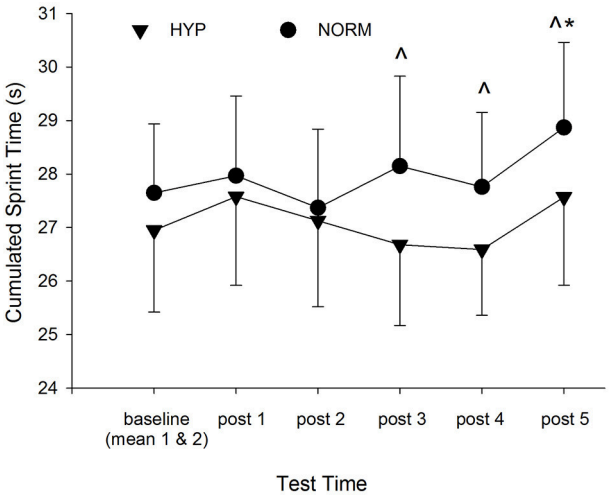

FIGURE 4 | Mean cumulated sprint time at baseline (mean of test 1 and 2) and the 5 post-intervention tests for the hypoxic (HYP) and normoxic (NORM) groups. *Substantially different from baseline. ^Substantially different between groups at each time point.

the HYP group improved cumulative sprint time compared to baseline at post-intervention 3 and 4 (1.0 ± 4.5 and 1.3 ± 4.5%, respectively, mean ± 90% CI, **Figure 4**). In comparison, over the same period the NORM group increased cumulative sprint time compared to baseline (1.7 ± 4.1 and 0.4 ± 4.4% for post-test 3 and 4, respectively). By post-intervention 5, both groups were slower over the 8 sprints compared to baseline (2.2 ± 4.2 and 4.3 ± 4.2% for the HYP and NORM groups, respectively). YYIR1 test performance improved throughout the study for both groups, with the effects of added hypoxia during repeat sprint training being unclear. Adding an extra two top-up sessions at the end of the 3-week post-intervention period had little beneficial effect in terms of further improving repeat sprint ability or YYIR1 performance in the HYP group over the next 2 weeks. Additionally, giving the NORM group two sessions of hypoxic training at the end of the study had little beneficial performance effect over the next 2 weeks (post 4 and 5). Standard deviations representing observed individual responses in performance in the post-exposure trials ranged from 1.7 to 2.8% for the repeat sprint ability test (using linear extrapolation) and 9.4–19.7% for the YYIR1 test. The typical error of the measurement for all participants between the two baseline tests was 0.8% (90% CL = 0.6–1.1%) and 4.7% (90% CL = 3.7–6.6%) for the repeat sprint ability and YYIR1, respectively.

DISCUSSION

The novel findings of this study were that six sessions of repeat-sprint training under hypoxia, simulating rugby match conditions, while otherwise living at sea-level, had long-lasting beneficial effects on sea-level repeat sprint ability in field conditions. In addition, off-feet training (using a cycle ergometer) was beneficial at improving on-feet performance (run-based repeated sprinting) in rugby players. Finally, it seems that six sessions (2 per week for 3 weeks) of hypoxic repeat-sprint training is necessary for improvements in repeat sprint ability and two sessions over 1 week, had little effect at eliciting further enhancement in performance in previously hypoxic trained athletes or athletes new to hypoxic training.

The error of measurement in this study was similar to measures from previous studies ∼1–2% for repeat sprints in team-sport athletes (Wood et al., 2006; Hamlin et al., 2012) and indicated good reliability of measures. Individual responses to hypoxia tended to be relatively small for repeat sprint ability and slightly larger for the YYIR1 test.

The hypoxic group in the present study had an ∼2% greater improvement in their repeat sprint ability compared to the normoxic group over the last 3 testing trials (**Table 3**), which compares favorably to repeat-sprint training derived improvements in previous hypoxic studies (Jones B. et al., 2015; Brocherie et al., 2015b). In association with less fatigue in the HYP group, these players were also faster over the 8 sprints during this period compared to the NORM group and were ∼1% faster at post-testing times three and four compared to baseline. However, not all researchers have found beneficial effects when adding hypoxia to repeat-sprint training

(Galvin et al., 2013; Goods et al., 2015). We suggest the cause of some of the conflicting results are likely to be associated with the mismatch between hypoxic training protocols and subsequent performance testing procedures. For example, Galvin et al. (2013) had rugby players complete 12 sessions of ten 6 s all out sprints with a 30 s recovery on a non-motorized treadmill under hypoxic conditions, but used a performance test that did not match this protocol (i.e., 10 × 20 m running sprints with 30 s rest period). Data derived from the Galvin et al. (2013) study shows that the average time to run 20 m by these athletes was ∼3 s. Therefore, to match the training program, these athletes should have been running ∼40 m during performance testing. Such details are important, as during training, these athletes would have been stressing and subsequently adapting, more aerobic than anaerobic metabolic processes. But during testing, these athletes would have been relying to a greater extent on anaerobic rather than aerobic metabolic systems. Indeed, these authors reported substantial improvements in endurance (aerobic) performance after training (∼15% improvement in hypoxic compared to normoxic group), which suggests the training was more conducive to endurance performance than anaerobic repetitive sprinting performance adaptations.

In contrast, Goods et al. (2015) used similar training and testing procedures to our study but found non-significant changes between normoxic and hypoxic groups post-exposure for repeat sprint running ability. These results conflict with the present study, which showed substantially improved repeat sprint running ability post hypoxic exposure. However, these researchers did not quantify the training loads between groups, which could possibly result in different training stress and therefore adaptation in the groups studied.

The different methods used to quantify the change in repeat sprint ability between studies may also influence results. A recent article suggests using the average or cumulated sprint time overcomes the problem of high variability associated with calculating fatigue scores from the first and last sprints in a set (Oliver, 2009). However, we have found that using a linear function to predict the first and last sprints of the log-transformed data, which can then be subtracted to give a percent fatigue index, can also produce meaningful fatigue values with a low co-efficient of variation (∼1%). We acknowledge that using this approach may overestimate the final sprint in a set which can be slightly faster due to participants inadvertently pacing themselves (Glaister et al., 2008), however, we found substituting the predicted 8th sprint with the actual recorded 8th sprint had little effect on the results. Indeed, when we calculated fatigue using the method suggested by Glaister et al. (2008) which can be found in **Table 3** under "Repeated sprint fatigue[2]," we found similar results to the linear extrapolation method.

Performance response to repeat-sprint training under hypoxic conditions is variable and inconsistent. While this study and others (Faiss et al., 2015; Kasai et al., 2015; Brocherie et al., 2015a,b) have shown beneficial changes in repeat sprint ability after repeat-sprint training in hypoxia compared to normoxia, this positive result is not always found (Goods et al., 2015; Montero and Lundby, 2016). Methodological differences including participant characteristics and ability, motivation and encouragement during training and testing, selection, and timing of performance tests and time, degree and frequency of hypoxia may theoretically help explain this variability in performance change. More research investigating these variables may help reduce performance variation with such training.

Postulated mechanisms associated with improved repeat sprint ability after repeat-sprint training in hypoxia include augmented anaerobic glycolytic metabolism (Svedenhag et al., 1991), improved acid-base homeostasis (Nummela and Rusko, 2000) and increased muscle blood perfusion (Faiss et al., 2013b). The increased repeat sprint ability in the hypoxic compared to the normoxic group in this study along with no substantial between group change in the aerobic measure (YYIR1) suggests improvement in the anaerobic rather than the aerobic metabolism is involved. However, this remains speculative since no mechanistic variables were measured in this study.

Generally the addition of hypoxia during exercise results in substantially higher ratings of perceived exertion (Shephard et al., 1992; Buchheit et al., 2012; Goods et al., 2014). It seems that when exercising under hypoxic conditions, even when the workload is reduced to account for the lowered oxygen availability (Buchheit et al., 2012), participants perceive the exercise to be more difficult. Some of this increased effort is probably due to the increased ventilator drive required during hypoxic exercise (Katayama et al., 2001), but increased peripheral muscular sensation via accumulation of hypoxic metabolites (Hogan et al., 1999) is probably also involved. Exercise under hypoxic conditions can also have a negative effect on cerebral oxyhemoglobin levels (Monroe et al., 2016) which may affect sensations directly. The increased perceived effort reported by the athletes in this study when adding normobaric hypoxia to high-intensity exercise (average training RPE increased from 14.7 to 15.6 in the hypoxic group compared to 13.9–15.5, in the normoxic group over the eight training sessions) is similar to previous research (Aliverti et al., 2011), but is in contrast to a recent study (Brocherie et al., 2016). Brocherie et al. (2016) found a slow reduction in the perceived effort reported by elite field hockey athletes as they completed six sessions of repeat-sprint training (four sets of five reps of 5 s running sprints in FIO2 ∼14.5%) over 2 weeks (average training RPE decreased from 14.6 to 13.1 in the hypoxic group and increased from 14.4 to 14.8 in the normoxic group over six training sessions). Differences between studies may be due to the exercise mode (cycling compared to running), the caliber of athletes (elite field hockey players compared to well-trained rugby players), or the fact that athletes performed the training while resided at a simulated altitude of ∼3000 m (Brocherie et al., 2016). In addition, the current study design with an increased resistance during training days 3 and 4 and a 3-week break between intervention 1 and 2 probably did not allow for such acclimation to be studied as rigorously as Brocherie's study.

A limitation to the current study was the fact that when participants were exercising under hypoxia the training stress was substantially higher compared to participants completing the same exercise under normoxic conditions (as witnessed by the significantly higher training heart rates and perceived exertion,

Figure 2). Therefore, we need to be careful when attributing the benefit of such training to hypoxia, since the performance benefits may simply be due to the harder training load in the hypoxic group. Designing a study that could control for training workload in the hypoxic and control groups would be one way to tease out this effect. A further limitation of this study is that we used well-trained rugby players in this study and therefore the results may not reflect what may occur in elite rugby players. Lastly this study was a field study rather than a lab-based study in order to improve the ecological value of the outcomes, however such studies also increase the risk of elevating variability due to extraneous variables. One such variable was the fact that all the rugby players in this study were required to play a pre-season game of rugby on the Saturday prior to the last testing day, which was probably responsible for the poor repeat sprint ability on the last testing day.

Finally, the present study found that "off-feet" repeat sprint cycling had a substantially beneficial effect on "on-feet" repeat sprint running ability in amateur rugby players. Such cross-over effects in performance enhancement is not always found in such studies (Goods et al., 2015), but indicates a potentially useful training protocol for athletic teams that have heavy on-feet training loads such as running. Currently the professional rugby season in New Zealand spans 9–10 months of the year (early February through to early November) which represents a large training load on players. Any opportunity to relieve on-feet training stress without reducing overall performance would be a useful supplementation to such athletes, however this research was conducted on amateur rugby players and would need to be replicated on professional players before firm recommendations for this group could be made.

AUTHOR CONTRIBUTIONS

MH conceptualized and designed the study, MH, PO, HM, assisted in the planning and acquisition of data, MH, PO, HM, CL, CE helped with the analysis and interpretation of the data, critically revising the manuscript and adding important intellectual content. All authors gave approval for the final version of this manuscript to be published and agree to be accountable for all aspects of the work.

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Heavy Resistance Training in Hypoxia Enhances 1RM Squat Performance

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Purpose: To determine if heavy resistance training in hypoxia (IHRT) is more effective at improving strength, power, and increasing lean mass than the same training in normoxia.

Methods: A pair-matched, placebo-controlled study design included 20 resistance-trained participants assigned to IHRT (FIO2 0.143) or placebo (FIO2 0.20), (n = 10 per group). Participants were matched for strength and training. Both groups performed 20 sessions over 7 weeks either with IHRT or placebo. All participants were tested for 1RM, 20-m sprint, body composition, and countermovement jump pre-, mid-, and post-training and compared via magnitude-based inferences.

Presentation of Results: Groups were not clearly different for any test at baseline. Training improved both absolute (IHRT: 13.1 ± 3.9%, effect size (ES) 0.60, placebo 9.8 ± 4.7%, ES 0.31) and relative 1RM (IHRT: 13.4 ± 5.1%, ES 0.76, placebo 9.7 ± 5.3%, ES 0.48) at mid. Similarly, at post both groups increased absolute (IHRT: 20.7 ± 7.6%, ES 0.74, placebo 14.1 ± 6.0%, ES 0.58) and relative 1RM (IHRT: 21.6 ± 8.5%, ES 1.08, placebo 13.2 ± 6.4%, ES 0.78). Importantly, the change in IHRT was greater than placebo at mid for both absolute [4.4% greater change, 90% Confidence Interval (CI) 1.0:8.0%, ES 0.21, and relative strength (5.6% greater change, 90% CI 1.0:9.4%, ES 0.31 (relative)]. There was also a greater change for IHRT at post for both absolute (7.0% greater change, 90% CI 1.3:13%, ES 0.33), and relative 1RM (9.2% greater change, 90% CI 1.6:14.9%, ES 0.49). Only IHRT increased countermovement jump peak power at Post (4.9%, ES 0.35), however the difference between IHRT and placebo was unclear (2.7, 90% CI –2.0:7.6%, ES 0.20) with no clear differences in speed or body composition throughout.

Conclusion: Heavy resistance training in hypoxia is more effective than placebo for improving absolute and relative strength.

Keywords: hypoxia, resistance training, strength, 1RM squat, power, hypertrophy

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INTRODUCTION

Hypoxia has long been used in combination with endurance training (Terrados et al., 1988). The benefits of endurance training in hypoxia include increased intermittent running performance (Inness et al., 2016), glycolytic enzyme activity (Faiss et al., 2013), and rates of phosphocreatine regeneration (Holliss et al., 2013). Hypoxia may also improve performance in time trials

(Czuba et al., 2011), although this is less conclusive (Inness et al., 2016). These benefits are potentially important to enhance team-sport athlete performance.

The development of strength and power is also important for team-sport athletes due to the physical involvements in collision sports including Australian football and the Rugby codes (Dawson et al., 2004; Roberts et al., 2008). Therefore, resistance training plays an integral part in the physical preparation of team-sport athletes. The positive effects of resistance training in hypoxia are becoming evident (Takarada et al., 2000b). When used in combination with resistance training, hypoxia is commonly achieved in two ways, blood flow restriction, and intermittent hypoxic resistance training (IHRT) With blood flow restriction, a pressure cuff is applied to the limb, restricting blood flow, participants then perform resistance training exercises (Takarada et al., 2000b). Blood flow restriction causes many perturbations in the muscle, only one of which is hypoxia. Restricting blood flow to the muscle places the restricted tissue in an ischemic state, leading to hypoxia. Some of the possible mechanisms behind increased strength and hypertrophy through blood flow restriction include increased type ii fiber type recruitment (Yasuda et al., 2010), accumulation of metabolites (Loenneke et al., 2010), increases in plasma growth hormone (Takarada et al., 2000a), and muscle cell swelling (Loenneke et al., 2012).

There are practical limitations to blood flow restriction when using traditional resistance training exercises such as squats, deadlifts, and bench press. Firstly, it is only possible to restrict blood flow to the limbs; therefore a large proportion of musculature used during these exercises is not in a hypoxic state. This causes a disassociation in muscle hypertrophy between the muscles of the limb, which is exposed to blood flow restriction, and the muscles of the trunk, which is not exposed to blood flow restriction (Yasuda et al., 2011). Since blood flow restriction is usually matched with low intensity resistance training, the changes in cross sectional area and strength may not be accompanied by a concomitant increase in connective tissue strength. This is due to decreased mechanical loading through low intensity resistance training used with blood flow restriction (Scott et al., 2015a), therefore the strength of muscles and connective tissue will adapt disproportionately. Increased tensile strength of the tendon might be expected to maintain the safety of the tendon under increasing loads (Buchanan and Marsh, 2002). Although currently unknown, there is a possibility that increasing the strength of the muscle without allowing the tendons time to adapt to this increased force production of the muscle may result in an increased risk of musculotendinous injuries. Due to these limitations in blood flow restriction, it is possible IHRT may be more suited for athletes and strength-trained individuals, as it allows high force production during training that also strengthens connective tissue to aid in injury prevention.

With IHRT, participants perform resistance training in hypoxia, induced by either a normobaric reduction of oxygen content in the mixture (Friedmann et al., 2003; Nishimura et al., 2010), or decreased partial pressure of air as evident at moderate to high altitude (Feriche et al., 2014). There is very little research on IHRT, with conflicting findings on its the effectiveness for increasing 1RM. One of the earliest studies using IHRT showed increased strength and hypertrophy compared to the same training in normoxia (Nishimura et al., 2010). For two sessions per week for 6 weeks, French press and arm curls were performed at 70% 1RM. The IHRT group showed greater hypertrophy and strength than the control group. To further support the use of IHRT, netball athletes undertaking low intensity IHRT had a greater improvement in maximal voluntary contraction than a control group (Manimmanakorn et al., 2013). Similarly, in previously untrained participants, using a moderate intensity resistance training protocol, only the IHRT group improved muscular endurance as measured by maximal repetitions at 70% 1RM (Kon et al., 2014). However, the IHRT group showed no greater improvement in 1RM compared to control (Kon et al., 2014). This is interesting considering a moderate training intensity of 70% 1RM, using 5 sets of 10 reps was used throughout the study.

In the aforementioned study, both groups had similar increases in lean mass and decreases in fat mass (Kon et al., 2014). A low intensity IHRT protocol with previously untrained participants did not have the same effect on muscular endurance (Friedmann et al., 2003). Maximal strength, as measured through a maximal voluntary contraction, did not increase. It is clear the current research is conflicting, and further investigations are required to deduce the best training combinations.

There is currently no research using a high intensity resistance training protocol. The effects of IHRT on strength-trained participants have also not been determined. This study will therefore investigate if heavy IHRT is more effective at developing maximal strength in resistance-trained participants. A secondary aim is to determine whether IHRT aids changes in body composition, sprint performance, and power production during the countermovement jump.

METHODS

Participants

Twenty strength-trained male participants aged between 18 and 34 volunteered as participants. Participants were required to record a training diary, and qualified as strength trained by achieving at least 12 months continuous resistance training history immediately prior to the study. Resistance training prior to the study needed to include squats and deadlifts as part of their regular training program. The study was approved by the University Human Research Ethics Committee and conformed to the Declaration of Helsinki. All participants provided written informed consent. Participants completed a training log of their current resistance training, including frequency, exercises, sets, repetitions, and intensity to ensure they qualified as strength trained for the purpose of the study. Participants were then pair-matched on absolute 1RM squat strength and training history, and assigned to either the hypoxic (IHRT) or placebo groups.

Testing

Pre-, mid-, and post-study, participants were scanned using dual energy x-ray absorptiometry (DXA) to assess changes in body

composition, tested for 1RM squat strength, countermovement jump, and 20-m sprint time with 5 and 10-m splits.

All participants were familiar with the 1RM squat, a warm up set of five repetitions at 50% predicted 1RM was performed, followed by three repetitions at 80% predicted 1RM, then a single repetition at 90% predicted 1RM. The weight was then increased in small increments until failure, with the goal of achieving a 1RM in a further 3–4 attempts. For the lift to be successful, a depth was required whereby the crease of the hips was below the top of the patella. The same Australian Strength and Conditioning Association Level 3 qualified coach assessed 1RM depth throughout the study. A linear position transducer was connected to the bar, and during the warm-up sets, participants were instructed as to the required depth for 1RM testing by the assessor. This depth was recorded via minimum displacement from the linear position transducer. A failed attempt was recorded if the participant failed to lift the weight, or if adequate depth was not achieved.

The 20-m sprint was performed pre- and post-intervention on the same indoor basketball court using Swift timing gates (Swift, http://www.spe.com.au. Wacol, Queensland, Australia). Participants were instructed to place their toe on a line between the two starting gates, with their weight over their front foot to ensure acceleration occurs from a stationary start with no rock back. Timing gates were placed at 0, 5, 10, and 20-m to record split times. A minimum of three attempts was allowed, with a fourth attempt given if the third trial was the fastest. Participants were offered as much rest as required to ensure each effort was maximal, with a minimum of 3 min given.

A countermovement jump using a force plate, linear position transducer, and corresponding Ballistic Measurement System Software (Fitness Technologies, http://www.fittech.com.au, Adelaide, South Australia, Australia) was used to assess jump qualities. The force plate and linear position transducer were calibrated prior to each testing session according to manufacturer's instructions. Participants performed a familiarization session for the countermovement jump prior to baseline testing. At each testing session, participants performed four single jumps with ∼30 s between each jump, with the jump that achieved the highest power output being used for analysis.

Body mass was measured on calibrated scales and height was measured on a calibrated stadiometer. A DXA scan was performed to assess change in body composition measures including fat mass, lean tissue, and bone mass. The DXA scanner used was a Discovery W version 13.4.2. It was calibrated prior to each testing session according to manufacturer's instructions. A standard scanning protocol was used to ensure measurement reliability (Nana et al., 2013). Two experienced technicians performed the scans throughout the study, however each participant was scanned and analyzed by the same technician at pre, mid, and post to remove any inter-tester differences. The protocol for the DXA scan is described in detail elsewhere (Nana et al., 2013). Briefly, participants were positioned on their back in the supine position with hands pronated and legs positioned slightly apart with the femur rotated inwards.

Participants were instructed to maintain a food diary for the first week of the study, and told to replicate this eating plan as closely as possible for the duration of the study. This was to minimize the likelihood that any changes in body composition were due to a change in diet. Participants were instructed not to start taking any supplements.

Training

During the training sessions, all participants wore a face-mask connected to a hypoxic simulator (Altitude Training Systems, http://www.ats-altitude.com, Lidcombe, NSW, 2141). The hypoxic simulator exposed the participants to simulated altitude by increasing the percentage of nitrogen in the inspired air. The simulator was set at one of two altitudes. The placebo group was exposed to air with an FiO_2 of 0.20, simulating an altitude of 400-m above sea level, while the hypoxic group was exposed to air with an FiO_2 of 0.145 for the first 4 weeks, simulating an altitude of 3100-m, and FiO_2 of 0.141 for the last 3 weeks, simulating 3400-m above sea level. Groups were blinded to their group allocation until the completion of all testing post-study. Participants completed 7 weeks of heavy resistance training three times per week, with sessions performed on non-consecutive days. Each session consisted of squats, deadlifts, and lunges, with repetitions ranging from 3 to 6, and sets ranging from 2 to 4 (**Table 1**). Rest periods were set at 3 min throughout the study. For squats, starting weight was 75% 1RM for session 1. This weight was chosen after pilot testing, as it was the heaviest weight that could be lifted whereby participants remained blinded to the simulated altitude. During pilot testing, when loads above 75% were used for six repetitions participants were able to correctly guess whether they were in the IHRT or placebo trial. We wanted to ensure participants remained blinded to their groups while still using heavy loads. The placebo effect may play a part in determining endurance changes due to altitude (Lundby et al., 2012), however it is unknown if the placebo effect plays a part in resistance training strength changes through altitude. To control for a possible placebo effect, it was important participants remained blinded to the group allocation. The starting weight for deadlift was the same as squat, while lunge started at 50% squat 1RM. If participants lifted the weight to the predetermined repetition goal, they were encouraged to increase the weight. Participants were asked for a rating of perceived exertion (RPE, Borg 6-20 scale) immediately post-set, and this, combined with the judgment of the researcher was used to determine whether the participant should increase the weight for the next set. This method of increasing the load each session was chosen over a set increase per session or week to allow for individual variations in adaptation over time. For squats and lunges, the bar was positioned on the back across the superior trapezius, with participants instructed to achieve a depth of crease of hips below the top of the patella, as for 1RM testing. For deadlift, the weight was lowered to the ground between each rep. As participants were experienced in these exercises, a degree of flexibility was allowed regarding placement of feet, with some choosing a wider stance, and others a narrower stance.

The hypoxic mask was worn for the last warm up set and all working sets. Once applied, the hypoxic mask was not removed until the completion of the session (∼45 min).

TABLE 1 | Repetitions (Reps) and sets each week for squats, deadlifts, and lunges (3 lunges each leg per set) and percentage 1RM for squat lifted and post-set RPE for each group each week.

Week	Sets	Squat reps	Deadlift reps	Lunge reps	Squat %1RM IHRT load	Squat %1RM placebo load	Post-set IHRT RPE	Post-set placebo RPE
1	3	6	6	6	77.5 ± 3.7	78.2 ± 3.9	6.74 ± 1.63	6.02 ± 2.19
2	3	5	5	6	85.4 ± 5.3	83.8 ± 4.0	7.39 ± 2.14	7.03 ± 1.90
3	3	4	4	6	94.4 ± 5.6#	90.5 ± 4.1	7.80 ± 2.28	7.38 ± 2.13
4	2	4	4	6	99.2 ± 5.4#	94.2 ± 4.5	6.98 ± 2.44	6.71 ± 2.16
5	3	5	5	6	100.4 ± 6.5#	94.7 ± 5.9	8.14 ± 2.49	7.66 ± 2.10
6	4	4	4	6	104.2 ± 7.8#	98.6 ± 6.5	7.86 ± 2.80	7.71 ± 1.87
7	4	3	3	6	109.9 ± 8.2#	104.5 ± 5.9	7.60 ± 2.80	7.83 ± 2.15

#*Likely Large effect in the difference of 1RM lifted between groups. All data is Mean ± SD.*

Prior to hypoxic exposure, baseline SpO_2 and heart rate were taken via pulse-oximetry. These values were also taken pre- and post-each working set, with the values immediately prior to the set used as the pre-set value, and the lowest value of SpO_2 and highest value for heart rate used as the post-set value. Post-set values were usually achieved 15–30 s post-set. As well as the 6–20 Borg scale post each working set, the Borg 1–10 RPE scale was used post-session. After the first session, and regularly throughout the 7 weeks, participants were asked which group they thought they were in.

Statistical Analysis

A contemporary statistical approach was used to analyse all data, and expressed as mean ± *SD* and effect size [ES ± 90% confidence limits (CL)]. Percentage change was determined in comparison to baseline. The difference in the change between groups was determined using both ES and % changes ± 90% CL. Where the difference in between group change for 90% CL crossed from positive to negative (across 0%), this was interpreted as unclear at 90% CL. Standards for measuring ES were as previously described (Hopkins et al., 2009).

RESULTS

Participants were blinded to condition, with only one participant guessing their group allocation. **Table 2** gives details of the participants. Of the 20 volunteers, 18 completed the study (9 per group), while the other two completed the mid testing. One participant withdrew due to illness, and the other through injury, both unrelated to the training study. Only the 18 who finished the study were included for post-analysis, while all 20 participants were included in the mid testing analysis. Groups were not clearly different for any of the testing procedures at baseline.

Baseline

Absolute strength was 121.4 ± 22.1 kg for IHRT and 125.5 ± 30.7 kg for placebo. Relative strength was 1.46 ± 0.19 $kg.bm^{-1}$ for IHRT and 1.56 ± 0.30 $kg.bm^{-1}$ for placebo. Differences between groups for strength, or any other testing parameter at baseline were unclear (**Table 2**).

Training Data

Pre-training SpO_2values were not different between groups (98.3 ± 1.3% for IHRT and 98.4 ± 1.3% for placebo; **Table 3**). During the session, oxygen desaturation occurred in IHRT only, with a *likely* large effect in the difference between groups pre-set (90.0 ± 2.5% for IHRT vs. 97.3 ± 1.3% for placebo. ES −5.61, 90% CL for ES −5.7 to −5.5). There was also greater desaturation post-set for IHRT with a *most likely* large effect in the difference between groups (84.1 ± 3.5% for IHRT vs. 96.5 ± 1.7% for placebo, ES −7.3, CL for ES −7.4 to −7.2; **Table 3**). Neither session RPE (IHRT 7.5 ± 1.3 vs. placebo 7.3 ± 1.6), nor post-set RPE (IHRT 15.4 ± 2.5 vs. placebo 15.2 ± 2.3) were different between groups.

Heart rate was higher post-set compared to pre in both groups (pre- to post-set HR 103.7 ± 19.6 to 144.8 ± 19.0 bpm for IHRT, and 104.0 ± 18.3 to 144.9 ± 18.2 bpm for placebo), with no clear difference between groups (**Table 3**). Load lifted when reported as percentage of squat 1RM was not different between groups during week 1 (77.5. ± 3.7% for IHRT vs. 78.2 ± 3.9 for placebo). For squat, there was a trend toward IHRT lifting a greater percentage of baseline 1RM compared to placebo throughout the study (**Table 3**). By week 3, IHRT was lifting a greater percentage of starting 1RM for squat compared to placebo, with a *likely* large effect (3.9%, ES 0.94, 90% CL 0.65–1.24). This greater percentage of 1RM lifted in training for IHRT compared to placebo remained through to week 7 (5.4%, ES 0.91, 90% CL 0.61–1.20).

Mid

Compared to baseline, both groups improved both absolute (12.7 ± 3.9% for IHRT vs. 8.7 ± 4.7% for placebo) and relative (12.9 ± 5.1% for IHRT vs. 8.2 ± 5.3% for placebo) 1RM squat (**Figure 1**). Compared to placebo, the IHRT group had a *possibly* greater change for absolute (4.4% greater change, CL 1.0–8.0%, ES 0.21, 90% CL 0.05–0.37) and a *likely* greater change for relative 1RM (5.1% greater change, CL 1.0–9.4%, ES 0.31, 90% CL 0.06–0.57). There was no change for lean mass or countermovement jump parameters either between or within groups.

Post

Compared to baseline both groups improved both absolute (22.2 ± 7.6% for IHRT vs. 14.2 ± 6.0% for placebo) and relative

TABLE 2 | Testing results for all performance tests for both IHRT and Placebo groups.

Test	IHRT group			Placebo group		
	Pre ($n = 10$)	Mid ($n = 10$)	Post ($n = 9$)	Pre ($n = 10$)	Mid ($n = 10$)	Post ($n = 9$)
Height (cm)	183.1 ± 4.5	183.1 ± 4.5	184.1 ± 4.5	181.0 ± 5.8	181.0 ± 5.8	180.8 ± 5.8
Body mass (kg)	83.1 ± 7.5	83.0 ± 7.5	83.7 ± 7.9	80.2 ± 12.0	80.5 ± 11.2	78.7 ± 9.8
Absolute 1RM strength (kg)	121.4 ± 22.1	138.2 ± 27.8	148.4 ± 32.7**	125.5 ± 30.7	135.7 ± 29.5	141.8 ± 28.8#
Relative squat strength ($kg.bm^{-1}$)	1.46 ± 0.19	1.66 ± 0.22	1.76 ± 0.26**	1.56 ± 0.30	1.69 ± 0.29	1.80 ± 0.25#
5 m split time (s)	1.17 ± 0.08		1.17 ± 0.03	1.13 ± 0.08		1.12 ± 0.07
10 m split time (s)	1.94 ± 0.11		1.94 ± 0.06	1.89 ± 0.09		1.88 ± 0.08
20 m split time (s)	3.25 ± 0.15		3.27 ± 0.10	3.21 ± 0.14		3.18 ± 0.09
Absolute Peak Power (W)	4360 ± 602		4676 ± 463*	4333 ± 656		4299 ± 527

*Possibly greater pre-post change than placebo. **Likely greater change than placebo. #Likely greater than pre. All data is Mean ± SD.

TABLE 3 | Average SpO_2 and Heart Rate values pre and post all sets for the duration of the training study and post-set RPE for each group.

	IHRT pre-session	IHRT pre-set	IHRT post-set	Placebo pre-session	Placebo pre-set	Placebo post-set
SpO_2%	98.29 ± 1.3	89.97 ± 2.5*	84.13 ± 3.5*#	98.38 ± 1.3	97.26 ± 1.3	96.51 ± 1.7
Heart Rate (BPM)	93.8 ± 19.1	103.7 ± 19.6	144.8 ± 19.0**	93.8 ± 17.2	104.0 ± 18.3	144.9 ± 18.2**
RPE (au)			15.43 ± 2.5			15.16 ± 2.3
Post-session RPE (au)			7.53 ± 1.28			7.27 ± 1.61

*Most likely less than placebo. #Most likely less than pre. **Most likely greater than pre-set. All Data is Mean ± SD.

(20.5 ± 8.5% for IHRT vs. 13.6 ± 6.4% for placebo). The IHRT group had a *likely* greater change than placebo from pre to post for both absolute (7.0% greater change, CL 1.3–13.0%, ES 0.33, 90% CL 0.06–0.59) and relative (8.0% greater change, 90% CL 1.6–14.9%, ES 0.49, 90% CL 0.10–0.87) 1RM. At post, only IHRT improved countermovement jump peak power, however this change was unclear in comparison to placebo (2.7% greater change, 90% CL −2.0–7.6%, ES 0.20, 90% CL −0.15–0.54). There was no difference between or within groups compared to pre for 20-m sprint or body composition.

DISCUSSION

Hypoxia improved both absolute and relative 1RM compared to the same training using a placebo despite no change in lean mass. The placebo group also improved 1RM strength, however the IHRT change was 5.8 and 9.2% greater than placebo in absolute and relative 1RM strength, respectively. Considering participants were already strength trained with a moderate to high level of strength, this is a meaningful change between groups after only 7 weeks.

This is the first study to use heavy resistance training combined with hypoxia. Only an acute resistance exercise bout has been previously used (Scott et al., 2015b), therefore the present study reports original findings using heavy resistance training and hypoxia in a training study. The majority of resistance and systemic hypoxia studies used low to moderate intensity resistance training of ∼30–70% 1RM. However, the present study used 75% 1RM for the first session, increasing to 104% starting 1RM for placebo and 109% for IHRT. Unlike high-intensity interval training in hypoxia, which shows a decrease in maximal work (Balsom et al., 1994), hypoxia does not appear to impact on physical performance during high-load resistance exercise. This is shown by no differences in RPE and heart rate between groups, and is in agreement with the only other research using heavy resistance exercise and hypoxia. Using five repetitions of squat and deadlift in three different conditions (FiO_2 of 0.21, 0.16, and 0.13), heart rate, and post-set RPE were not different in the conditions with an FiO_2 of 0.21 and 0.16 despite using the same load. However, at the more extreme hypoxia, heart rate was higher than the other two conditions (Scott et al., 2015b). This, combined with our findings, confirms resistance training exercise intensity is not affected by moderate hypoxia, at least in trained individuals.

While increased strength and hypertrophy is consistently achieved through resistance training combined with blood flow restriction (Takarada et al., 2000b; Scott et al., 2014), there are conflicting findings whether systemic hypoxia increases strength to a greater extent than resistance training alone. The inconsistent findings can be partly attributed to differences in study design. For example, studies of moderate volume and intensity (3–4 sets of ∼70% 1RM) generally show greater changes in muscle hypertrophy using hypoxia (Nishimura et al., 2010; Kurobe et al., 2015), although one showed no difference between groups (Kon et al., 2014). There are also inconsistent results regarding changes in 1RM between groups when using IHRT with submaximal loads. Studies show increases in 1RM using both moderate (Nishimura et al., 2010) and very light loads (Manimmanakorn et al., 2013), while others show no benefit on 1RM through hypoxia compared to control (Kon et al., 2014). Unfortunately, the study by Nishimura et al. used a predictive equation to determine 1RM from a 10RM test. Therefore, although authors concluded an increase in muscular strength, it is rather likely that, because of the non-specific testing (Reynolds

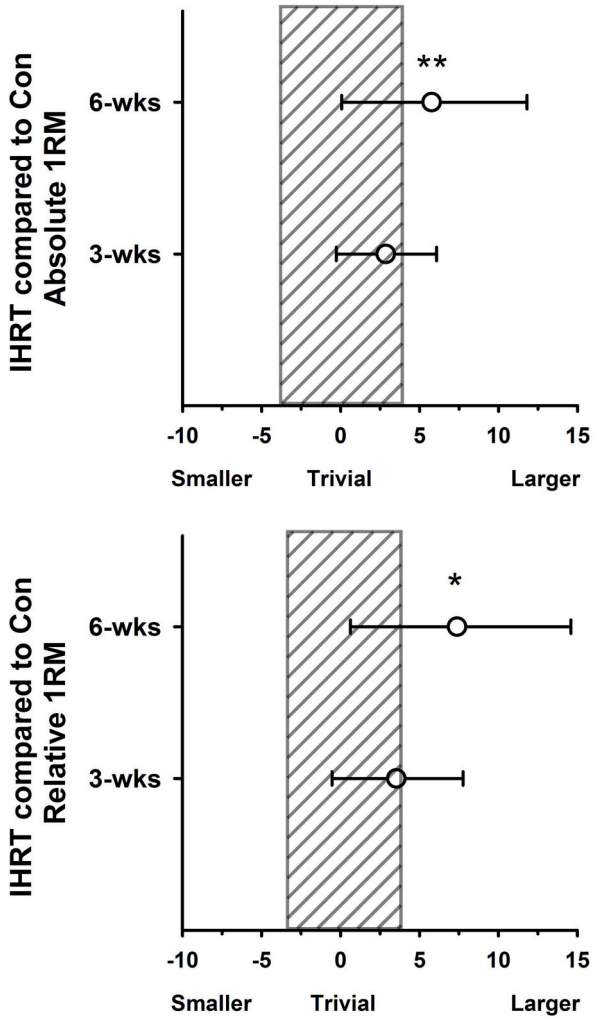

FIGURE 1 | Percentage difference in the change in performance from baseline for intermittent hypoxic resistance training (IHRT) compared with placebo with 90% CI. (A) Between-groups change in Absolute 1RM strength. **(B)** Between-groups change in Relative 1RM strength. Shaded bar represents uncertainty in the measure. **Possibly* small effect in the difference in change between groups, ***Likely* small effect in the difference in the change between groups.

et al., 2006; Tanner and Gore, 2013), an increase in muscular endurance explained the change in predicted 1RM.

Two studies have used tests of moderate intensities to test for muscular endurance, similar to the load used during training, with hypoxic groups showing both a benefit (Kon et al., 2014), and no change in performance (Kurobe et al., 2015). In the Kon et al. study, systemic hypoxia showed no greater change in 1RM compared to control, however when testing using the same intensity as training, muscular endurance improved more in the hypoxic group (Kon et al., 2014), showing the importance of the testing protocol matching the training protocol.

As all hypoxia and resistance training studies to date have used moderate intensities more suited to hypertrophy and muscular endurance gains (Fleck and Kraemer, 2014), combined with the differences in methodology for determining 1RM, it is not surprising that changes in 1RM through systemic hypoxia are conflicting. Therefore, it is important to ensure the testing battery is appropriate to reflect the nature of the training intervention (Tanner and Gore, 2013). Systemic hypoxia may merely magnify the expected outcome from a given training program, with specificity of the stimulus and testing protocol important to test the outcome of training.

It is possible there were no increases in lean mass due to our study employing a lower volume, high intensity program designed to increase maximal strength more so than muscle hypertrophy. This type of protocol is more likely to see changes in strength as opposed to muscle hypertrophy (Kraemer and Ratamess, 2004). This change in strength despite a lack of change in lean mass is an important finding, as many athletes, including team-sport athletes with a high running demand in their sport, athletes competing in weight classes, and many endurance athletes want to increase strength without an increase in mass.

Other researchers have concluded that the placebo effect may be at least partly responsible for performance changes through hypoxia (Siebenmann et al., 2012), however this was not the case in our study. As participants were successfully blinded to the environmental condition, the greater changes in strength observed in IHRT in the present study cannot be attributed to a placebo effect. Due to no placebo effect, and no changes in lean mass, we cannot fully explain the mechanisms responsible for the differences between groups. When beginning strength training, most of the early strength changes can be attributed to neural adaptations (Moritani and deVries, 1979). Such early changes include adaptations in agonist, antagonist, and stabilizer muscle activation, increased firing frequency, motor unit synchronization, and agonist co-activation (Folland and Williams, 2007). As all participants were strength trained, matched for training status and 1RM, any neural adaptations could be expected to be minimal.

When undertaking endurance training in hypoxia, intermittent hypoxic training maintains the proportion of type II fibers to a greater extent to the same training in normoxia (Zoll et al., 2006). After 6 weeks, an intermittent hypoxic training group had the same percentage of type II fibers (29.4 $\pm$ 7.4 at pre, and 29.9 $\pm$ 6.2 at post), whereas the same training in normoxia saw type II fibers decrease (41.0 $\pm$ 8.1% at pre, to 33.9 $\pm$ 6.6% at post; Zoll et al., 2006). Type II muscle fibers have a greater force production than type I fibers (Bottinelli et al., 1999). As intermittent hypoxic training maintains type II fibers, if this maintenance of type II fibers is also apparent with IHRT, it is possible there was a greater strength adaptation in the IHRT group compared to placebo despite no changes in muscle mass due to type II fiber maintenance.

The decreases in SpO_2 in IHRT are similar to other systemic hypoxic studies (Kon et al., 2010; Scott et al., 2015b). A decrease in SpO_2 occurs with hypoxia, and this activates a cascade of events that eventually lead to changes in endurance performance (Rusko et al., 2004). It is yet to be determined what effect a decrease in SpO_2 has on changes seen through IHRT. There is reduced central fatigue following adaptation to hypoxia (Amann et al., 2013). Enhanced cerebral O_2 delivery to compensate

for hypoxia could enhance neurotransmitter turnover, thus enhancing skeletal muscle fiber firing rate (Amann et al., 2013), which is a typical neural adaptation seen through resistance training. The heavy resistance training used in the current study increases the level of neural activity (Tan, 1999). Whether these changes are evident following IHRT is unknown, however if apparent, this would possibly explain an adaptation to hypoxia that may increase strength through neural changes including muscle fiber firing rate. Although there are possible neural changes that could explain increases in 1RM through IHRT, most changes in neural adaptation occur quite early in a resistance-training program (Moritani and deVries, 1979). As we used resistance-trained participants, most of the neural changes would have already occurred. Therefore, it is quite surprising that there were changes in 1RM despite no change in muscle mass. Because of this, the mechanisms behind increased 1RM are not known. Neural changes should be analyzed in further IHRT studies to determine whether neural changes are responsible for the change in performance following IHRT.

We found no change in 20-m sprint performance for either group, despite a change in 1RM. Although only IHRT improved CMJ peak power, the difference in the change between groups was unclear. There is a strong correlation between maximal squat strength and sprint performance (Wisløff et al., 2004; McBride et al., 2009), this correlation is also apparent between maximal squat strength and CMJ performance (Wisløff et al., 2004). It is also generally believed that improving 1RM directly increases sprint performance. In soccer athletes, there was a small change in 20-m sprint times after improvements in 1RM squat strength (Styles et al., 2016). A low volume, heavy resistance training protocol was used. Although not stated, the Styles et al. study was performed during the competition season and it is assumed these athletes would have been exposed to maximal running velocity during training and matches. Therefore, strength changes through resistance training, combined with the sprint training during the on pitch training may have combined to increase 20-m sprint performance. To support this, strength training only, and sprint training only displayed the same changes in 30-m sprint times, while a combination of strength and sprint training had a greater enhancement in 30-m sprint times (Marques et al., 2015).

As our study was heavy resistance training only, and the participants were not performing sprint training as part of their normal activity outside of the study, a possible reason for the lack of change in 20-m sprint times and CMJ performance in our study is due to no explosive training being performed as part of training. In a study on team-sport athletes, a group that performed plyometric exercises improved sprint times, while a control group showed no change (Marques et al., 2013), however it should be noted that neither group performed resistance training as part of the study. The addition of sprint or plyometric training combined with maximal strength training may therefore be important to improve sprint times, with resistance training alone insufficient to increase speed and power.

In a normal periodized resistance-training program for athletes, high velocity resistance training either follows, or is completed in conjunction with heavy strength training. This change in strength could well be transferred into changes in power with appropriate subsequent training.

PRACTICAL APPLICATIONS

- IHRT increases relative and absolute 1RM in comparison to a strength and training matched control group.
- Increases in 1RM occurred despite no changes in muscle mass, 20-m sprint or CMJ parameters.
- Athletes wanting to increase strength without increasing muscle mass are advised to undertake heavy resistance training in systemic hypoxia.

AUTHOR CONTRIBUTIONS

MI, FB, RA responsible for study design, recruitment of participants, data collection, data analysis, manuscript drafting and editing. EW, AP, AS responsible for some data collection, some data analysis and editing manuscript.

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Sex-Specific Impact of Ischemic Preconditioning on Tissue Oxygenation and Maximal Concentric Force

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Prior peripheral hypoxia induced via remote ischemic preconditioning (IPC) can improve physical performance in male athletes through improved O_2 delivery and utilization. Since females may have an innate protective mechanism against ischemia-reperfusion injury, and since muscle metabolism during contraction differs between sexes, it is relevant to examine the impact of sex in response to IPC to determine whether it is also ergogenic in females. In a randomized, crossover, single-blind study, we investigated muscle performance, hemodynamic and O_2 uptake in strength-trained males ($n = 9$) and females ($n = 8$) performing five sets of 5 maximum voluntary knee extensions on an isokinetic dynamometer, preceded by either IPC (3 × 5-min ischemia/5-min reperfusion cycles at 200 mmHg) or SHAM (20 mmHg). Changes in deoxy-hemoglobin (Δ[HHb], expressed in percentage of arterial occlusion and considered an index of O_2 extraction), and total hemoglobin (Δ[THb]) concentrations of the vastus lateralis muscle were continuously monitored by near-infrared spectroscopy. The metabolic efficiency of the contractions was calculated as the average force/$\Delta[HHb]_{avg}$ ratio. Cohen's effect sizes (ES) ± 90% confidence limits were used to estimate IPC-induced changes and sex differences. IPC increased total muscular force in males only (13.0%, ES 0.64, 0.37;0.90), and this change was greater than in females (10.4% difference, ES 0.40, 0.10;0.70). Percent force decrement was only attenuated in females (−19.8%, ES −0.38, −0.77;0.01), which was clearly different than males (sex difference: ES 0.45, −0.16;1.07). IPC also induced different changes between sexes for average muscle O_2 uptake in set 2 (males: 6.4% vs. females: −16.7%, ES 0.21, −0.18;0.60), set 3 (males: 7.0% vs. females: −44.4%, ES 0.56, −0.17;1.29), set 4 (males: 9.1% vs. females: −40.2%, ES 0.51, −0.10;1.13), and set 5 (males: 10.2% vs. females: −40.4%, ES 0.52, −0.04;1.09). However, metabolic efficiency was not meaningfully different between conditions and sexes. IPC increased muscle blood volume (↑[THb]) at rest and during recovery between sets, to the same extent in both sexes. Despite a similar IPC-induced initial increase in O_2 delivery in both sexes, males displayed greater peripheral O_2 extraction and greater strength enhancement. This ergogenic effect appears to be mediated in part via an up regulated oxidative function in males. We conclude that strength-trained males might benefit more from IPC than their female counterparts during repeated, maximal efforts.

Keywords: blood flow restriction, muscle function, oxygenation, performance, sex differences, athletes

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INTRODUCTION

Ischemic preconditioning of a limb (IPC) is a non-invasive technique inducing transient peripheral hypoxia to subsequently enhance tissue tolerance against ischemia-reperfusion injury. This technique promotes local vasodilation, improves O_2 delivery (Enko et al., 2011; Bailey et al., 2012a), and enhances the efficiency of muscular contraction (Pang et al., 1995; Moses et al., 2005). Higher muscle O_2 utilization was also demonstrated in the quadriceps of strength-trained male athletes after performing IPC compared to placebo compressions (Paradis-Deschenes et al., 2016). It is not surprising, therefore, to observe that IPC can improve maximal physical performance in various exercise modes in male participants (De Groot et al., 2010; Bailey et al., 2012a; Paradis-Deschenes et al., 2016), although this is not a universal finding (Incognito et al., 2015). Before exercise sport performance applications, IPC was originally studied for its potential clinical relevance, and in this context, it is interesting to note that females appeared to display smaller clinical benefits compared with their male counterparts. In a cohort of 382 subjects, a failure to induce preconditioning effects during percutaneous coronary intervention was noted in females (Laskey and Beach, 2003), presumably due to the innate, multiple, protective actions of estrogens (Pitcher et al., 2005). Moreover, since physiological responses to exercise (in particular metabolic pathway utilization and perfusion) may differ between sexes, and that exercise performance data from females is almost inexistent, it is relevant and timely to examine the impact of IPC on females compared to males to more precisely evaluate the potency of this technique for exercise performance applications.

Females have been reported to exhibit less fatigue than males during intense exercise (Parker et al., 2007; Hunter, 2016), but not under conditions of ischemia (Russ and Kent-Braun, 2003), suggesting that muscle perfusion and oxygenation may be involved in the sex-related difference (Russ and Kent-Braun, 2003; Clark et al., 2005; Hunter et al., 2006). Indeed, females exhibit greater vasodilation in the limbs during single knee extensions (Parker et al., 2007), and may have a greater proportional area of type I fibers (Simoneau et al., 1985; Staron et al., 2000) and greater capacity for utilizing oxidative metabolism than males (Kent-Braun et al., 2002). Thus, females exhibit a greater reliance on oxidative metabolism compared with males, which could challenge the ergogenic impact of IPC.

Prior evidence examining sex-related differences in the response to IPC during exercise is limited. Gibson et al. (2015) reported no performance difference between male and female team-sport athletes during five repeated 6-s sprints after performing 3 × 5-min occlusions at 220 mmHg to both legs. However, the IPC procedure used in that study failed to improve performance in either sex, making the sex comparison moot. Another study demonstrated the positive impact of IPC (2 × 3-min at 220-mmHg) on recovery from squat jump test and running sprint performance 24 h after an initial, fatiguing session in males, but the female cohort was too small to draw any firm conclusion (Beaven et al., 2012). Considering the scarcity of studies and that none have attempted to measure relevant physiological responses to provide sex-specific mechanistic insights, any conclusion on the usefulness of IPC for female athletes cannot robustly be drawn.

The aim of the current investigation was therefore to determine the impact of IPC on muscle force and haemodynamics (blood volume and O_2 extraction) derived from near-infrared spectroscopy in males vs. females during repeated maximal efforts separated with incomplete recovery periods. We chose isolated contractions specifically to avoid confounding effects that inspiratory muscle fatigue can have on limb blood flow and O_2 uptake (Kayser et al., 1997).

MATERIALS AND METHODS

Participants

Strength-trained (power and weight lifters, cross-fit and taekwondo athletes) males ($n = 9$, age 25 ± 2 year; height 1.78 ± 0.02 m; weight 86.5 ± 4.9 kg) and females ($n = 8$, age 22 ± 1 year; height 1.66 ± 0.02 m; weight 60.8 ± 2.7 kg) volunteered to take part in this study. All performed 3–5 weight training sessions per week. All participants were non-smokers, free of health problems, did not use any medication, and were asked to avoid vigorous exercise, alcohol and caffeine 24 h before the tests. All but one female (who was amenorrheic) were tested in their follicular phase. Participants provided written informed consent after being informed of experimental procedures, associated risks and potential benefits. The study was approved by the Ethics committee of Université Laval, and adhered to the principles established in the Declaration of Helsinki.

Experimental Design

Participants visited the laboratory for one familiarization and two experimental trials. Resting heart rate and blood pressure (inclusion criteria <140/100 mmHg) were taken prior to every trial. During the first visit, height, weight and thigh circumference were measured. Thigh circumference (males: 61.3 ± 1.9 cm; females: 57.2 ± 1.9 cm) was measured by the same experimenter, 1 cm under the gluteal line. Participants then completed a familiarization session with the experimental set-up, comprising one 3-min compression at a pressure of 200 mmHg, and a standardized warm-up consisting of 5 min of cycling on a Monark ergometer (Ergomedic 828 E) at 100 W. The warm-up was continued with 3–5 right-leg extensions on an isokinetic dynamometer (Kin-Com 500 H, Chattecx Corp., Hixson, TN) at 20°/s, with effort perception progressing from 3 to 9 out of a scale of 10. After 2 min of rest while seated on the dynamometer, participants completed three complete sets of the exercise protocol described below.

Following familiarization, participants were randomized into IPC or SHAM groups in a single-blind, crossover design. In both conditions, participants were seated comfortably on a bed with both legs outstretched, and a non-elastic nylon blood pressure cuff (WelchAllyn, Skaneateles Falls, NY, USA, width: 21 cm) was positioned around the right upper thigh under the gluteal line. In IPC, the cuff was rapidly inflated to 200 mmHg for 5 min, and this was repeated three times with each compression episode separated by 5 min of reperfusion (cuff release) in the same position. A plateau in the NIRS-derived deoxy-hemoglobin

concentration signal (see NIRS procedure below) was observed in every subject by 5 min, and taken as a sign of effective occlusion and ischemia. In SHAM, the cuff was inflated to 20 mmHg. To minimize any placebo effect, participants were told that the purpose of the study was to compare the impact of two different cuff pressures that could both alter performance.

The familiarization session and experimental trials were separated by a minimum of 3 days to eliminate the potential effects of the second window of protection caused by IPC (Bolli, 2000), and a maximum of 7 days. All trials were performed at the same time of day for every participant to avoid potentially confounding circadian rhythm effects. The laboratory temperature was controlled and constant (20.31 ± 0.02°C) throughout all trials.

Exercise Protocol

The exercise protocol started 18.5 ± 0.1 min after the end of the last cycle of compression. Participants were seated in an upright position on the isokinetic dynamometer, and a strap was secured tightly across the pelvis. The right leg was fixed to the dynamometer with a strap above the ankle external malleoli, and the axis of rotation was aligned to the lateral femoral condyle of the knee joint.

The protocol consisted of five sets of 5 maximum voluntary knee extensions (60° range of motion from 80 to 20°; 0° corresponding to knee fully extended) at 20°/s angular velocity (one extension lasting ∼3.0 s). Participants were instructed to contract as hard as they could throughout the extension, and were strongly encouraged during all contractions. Contraction was stopped during flexion when the dynamometer arm automatically returned to 80° at angular velocity of 120°/s (lasting less than 0.5 s), and started immediately after the return of the arm. Subjects rested quietly and relaxed for 30 s between each set and after the last set. After the exercise, participants moved back to the bed to perform an arterial occlusion with the cuff at 200 mmHg (∼3–5 min) to obtain a physiological calibration of the NIRS signals. The cuff pressure was released after the deoxy-hemoglobin signal had reached a plateau (see Near-infrared spectroscopy procedure below). Participants were also asked which condition between IPC and SHAM they felt had the greatest impact on their performance, and their verbal answer recorded.

The force produced by participants was measured with a force transducer connected at the end of the level arm of the dynamometer, which was calibrated according to the manufacturer's recommendations before every trial (manufacturer typical error 0.5%). The intra- and inter-day coefficient of variation for force obtained by the main experimenter was 2.4%. Force signals were analyzed in Matlab® between a starting point defined when velocity was ≥18°/s, angle was ≥80° and force was ≥100 N, and an end point when velocity was ≥18°/s and angle was ≥20°. In every set, peak and average force were calculated. Total force was then calculated as the sum of the average force produced in all sets. Percent force decrement across all sets was calculated as follows: 100 − ([total force output/ideal force output] × 100), where total and ideal force outputs are the sum of average force values from all sets and the highest average force was multiplied by five, respectively.

Near-Infrared spectroscopy (NIRS)

NIRS is a versatile, non-invasive methodology providing semi-quantitative measures of tissue oxygenation, and is easily applied to study a variety of tissue regions in individuals. NIRS quantifies the changes in hemodynamics from changes in the absorption of near-infrared light by oxyhemoglobin (HbO_2) and deoxyhemoglobin (HHb) (Mccully and Hamaoka, 2000). With this technique, oxygenation can be measured in a discrete region of a tissue in a working physiological setting, which enhances specificity and has distinct advantages as compared to more cumbersome methods. Muscle tissue oxygenation measured by NIRS reflects the balance of O_2 delivery to working muscles and muscle O_2 consumption in capillary beds (De Blasi et al., 1993; Ferrari et al., 2004). Assessment of de- and re-oxygenation kinetics during and after dynamic exercise has become increasingly popular in recent years as a means to non-invasively assess the aerobic function of skeletal muscle. In the current protocol, muscle blood volume and oxygenation were assessed using a portable spatially resolved, dual wavelength NIRS apparatus (PortaMon, Artinis Medical Systems BV, Netherlands). The NIRS device was installed on the distal part of the right vastus lateralis belly (approximately 15 cm above the proximal border of the patella). Skinfold thickness was measured at the site of the application of the NIRS (males: 8.7 ± 0.9 mm; females: 10.7 ± 2.0 mm) using a Harpenden skinfold caliper (Harpenden Ltd) during the familiarization session, and was less than half the distance between the emitter and the detector (i.e., 20 mm). This thickness is adequate to let near-infrared light through muscle tissue (Mccully and Hamaoka, 2000). The skin was cleaned with an alcohol swab, and the device was fixed using double-sided stick disks and tape. Black bandages were used to cover the device to eliminate potentially interfering background light. The position of the apparatus was marked with an indelible pen for repositioning during the subsequent visit. The pressure cuff was positioned above the NIRS device, which did not affect the placement of the device during occlusions.

A modified form of the Beer-Lambert law, using two continuous wavelengths (760 and 850 nm) and a differential optical path length factor of 4.95, was used to calculate micromolar changes in tissue oxy-hemoglobin ($\Delta[HbO_2]$), deoxy-hemoglobin (Δ[HHb]) and total hemoglobin ($\Delta[THb] = [HbO_2] + [HHb]$; used as an index of change in regional blood volume). NIRS data were acquired at 10 Hz. At rest, once the signal was stabilized, 1 min of baseline values were analyzed pre IPC and SHAM treatments. Then, NIRS signals were analyzed 2-min post treatment for a duration of 1 min to assess the impact of IPC on resting blood volume ($\Delta[THb]_{rest}$, μM). During exercise, NIRS analysis was limited to Δ[HHb] since this variable is less sensitive than $[HbO_2]$ to perfusion variations and abrupt blood volume changes during contraction and recovery (De Blasi et al., 1993; Ferrari et al., 2004). The [HHb] signal was averaged over the last second of every contraction and over every set to obtain peak ($\Delta[HHb]_{peak}$, % arterial occlusion) and mean ($\Delta[HHb]_{avg}$, % arterial occlusion) O_2 extraction, respectively. These [HHb] data

were then normalized to express the magnitude of changes from baseline, and expressed in percentage of the maximal amplitude calculated during an arterial occlusion performed at the end of exercise. Contraction metabolic efficiency was calculated as the average force/$\Delta[HHb]_{avg}$ ratio. Finally, during recovery periods between exercise sets, the muscle reoxygenation rate (ΔReoxy, $\mu M.s^{-1}$) was calculated as the rate of change in [HHb] from the end of the exercise set to the end of the subsequent recovery period (i.e., the recovery of [HHb]; Billaut and Buchheit, 2013). During this period, the amplitude of change in [THb] ($\Delta[THb]_{rec}$) was also analyzed.

Statistical Analysis

All data are reported as means ± standard error (SE) or percentage changes from SHAM. The IPC-SHAM differences within the same group and between sexes were analyzed using Cohen's effect sizes (ES) ± 90% confidence limits (Batterham and Hopkins, 2006; Hopkins et al., 2009). Except for $\Delta[THb]_{rest}$ and $\Delta[THb]_{rec}$, all variables were log-transformed prior to analysis (Hopkins et al., 2009). Magnitudes of difference between conditions were determined with an effect size of 0.2 set to evaluate the smallest worthwhile change. Standardized effects were classified as small (>0.2), moderate (>0.5) or large (>0.8). The effect was deemed "unclear" if chances of having better/greater or poorer/lower change in performance and physiological variables were both >5% (Batterham and Hopkins, 2006; Hopkins et al., 2009).

RESULTS

All 17 participants met all criteria, completed the entire protocol, and tolerated the IPC procedure without complications. None of the participants could tell what condition produced the greatest change in performance.

Performance

Force parameters for males and females are displayed in **Table 1** and **Figures 1**, **2**. After the IPC manoeuver, total force clearly increased in males (13.0%, ES 0.64, 0.37;0.90), but the change was trivial in females (2.3%, ES 0.10, −0.17;0.38). &&&The sex difference for this parameter was clear (ES 0.40, 0.10;0.70; **Figure 1**). Specifically, the IPC-induced increase in average force was greater in males than females in every set of the protocol (set 1–males: 15.2% vs. females: 0.7%, ES 0.53, 0.16;0.90, set 2–males: 15.6% vs. females: 3.6%, ES 0.43, 0.10;0.76, set 3–males: 11.1% vs. females: 2.6%, ES 0.31, 0.02;0.61, set 4–males: 14.5% vs. females: 3.1%, ES 0.41, 0.07;0.76, set 5–males: 8.4% vs. females: 1.7%, ES 0.25, −0.06;0.56; **Figure 2**).

Importantly, although IPC did not increase average force in females, it clearly augmented 1-s peak force in sets 1–3 (**Table 1**), whereas males displayed benefits in sets 1, 2, 4, and 5. This effect was higher in males compared to females in sets 2 and 5.

Percent force decrement was attenuated in females after IPC (−19.8%, ES −0.38, −0.77;0.01), which was clearly different than males (sex difference: ES 0.45, −0.16;1.07).

Muscle Hemodynamics and Oxygenation

Physiological variables are displayed in **Table 2**. No difference was observed between IPC and SHAM in both groups for NIRS variables during baseline before the manoeuver. IPC increased $\Delta[THb]_{rest}$ to the same extent in males and females (males: 1.5% vs. females: 1.5%, ES 0.00, −0.80;0.79). After IPC, $\Delta[THb]_{rec}$ was increased in males after sets 1 (1.2%, ES 0.33, −0.14;0.80) and 5 (1.5% ES 0.44, 0.17;0.70). In females, $\Delta[THb]_{rec}$ was only enhanced after sets 2 (0.7%, ES 0.23, −0.09;0.54) and 3 (1.8% ES 0.54, 0.04;1.04). This change in $\Delta[THb]_{rec}$ was higher in males compared to females in set 5 only (sex difference: ES 0.48, 0.10;0.87). There was no change in ΔReoxy within the same group and between sexes.

TABLE 1 | Performance variables in IPC and SHAM conditions for males and females.

	Females			Males			Sex difference
	SHAM	IPC	% difference (ES) 90% CL	SHAM	IPC	% difference (ES) 90% CL	(ES) 90% CL
Peak force S1 (N)	449.1 ± 37.2	481.3 ± 42.1	7.2%, ES 0.24*, −0.07;0.56	627.9 ± 45.1	685.3 ± 35.7	10.2%, ES 0.41*, 0.23;0.59	ES 0.10, −0.24;0.44
Peak force S2 (N)	421.1 ± 32.8	446.3 ± 33.1	6.4%, ES 0.22*, 0.00;0.43	566.3 ± 31.3	648.9 ± 47.6	13.7%, ES 0.54*, 0.32;0.75	ES 0.24*, −0.03;0.50
Peak force S3 (N)	402.5 ± 22.7	429.5 ± 28.3	6.3%, ES 0.22*, 0.05;0.38	558.8 ± 35.7	581.6 ± 41.7	3.8%, ES 0.16, −0.01;0.32	ES −0.09, −0.29;0.12
Peak force S4 (N)	402.5 ± 24.7	422.0 ± 25.0	5.1%, ES 0.18, −0.06;0.41	526.3 ± 29.8	574.6 ± 43.2	8.4%, ES 0.34*, 0.06;0.61	ES 0.11, −0.20;0.42
Peak force S5 (N)	401.8 ± 27.1	397.5 ± 24.5	−0.7%, ES −0.02, −0.17;0.12	506.9 ± 28.3	544.0 ± 39.1	6.7%, ES 0.27*, 0.01;0.54	ES 0.26*, 0.00;0.51
Force decrement (%)	10.4 ± 1.5	8.4 ± 1.4	−19.8%, ES −0.38*, −0.77;0.01	13.7 ± 2.7	15.2 ± 2.2	6.0%, ES0.12, −0.54;0.77	ES 0.45*, −0.16;1.07

Values are mean ± SE.
Asterisks (*) denote "clear" effect sizes (see statistics section).

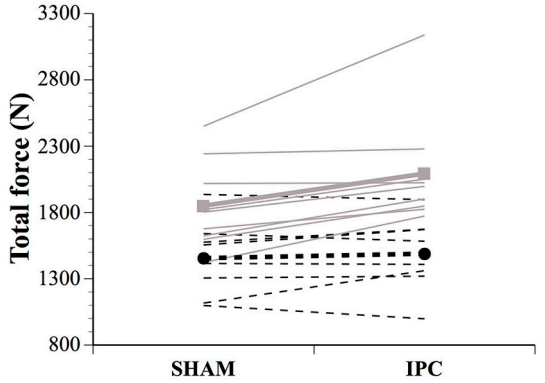

FIGURE 1 | Individual and average total force developed in SHAM and IPC conditions for males (■) and females (●). The IPC-induced change in total force was clearly higher in males than females. Values are mean ± SE.

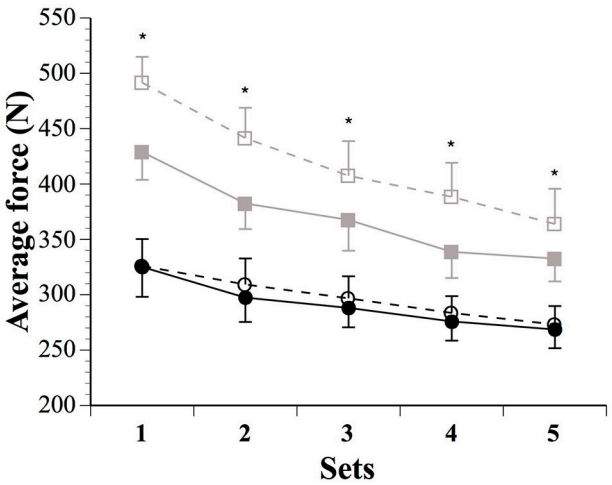

FIGURE 2 | Average force produced during the five sets in males (SHAM: ■ IPC: □) and females (SHAM: ● IPC: ○). Asterisks (*) denote "clear" differences between sexes (see statistics section). Values are mean ± SE.

The IPC maneuver did not alter muscle $\Delta[HHb]_{peak}$ across sets nor between sexes (**Table 2**). However, $\Delta[HHb]_{avg}$ were higher after IPC in males for set 1 (18.1%, ES 0.31, −0.11;0.73), and lower in females for sets 3 (−44.4%, ES −0.25, −0.61;0.11), 4 (−40.2%, ES −0.22, −0.52;0.08), and 5 (−40.4%, ES −0.22, −0.50;0.06). There was a clear sex difference in the impact of IPC on global muscle deoxygenation in sets 2–5 (**Figure 3**). The contraction metabolic efficiency ratio was not altered by IPC during the sets or between sexes (**Table 2**).

DISCUSSION

Summary of Main Findings

This study investigated the impact of sex on performance and vasoactive and oxidative responses to IPC in strength-trained athletes during repeated, maximal contractions. The main findings were that IPC (1) increased muscle force to a greater extent in males than females; (2) increased resting blood volume similarly in both sexes; and (3) increased O_2 extraction in males but decreased it in females. These results challenge the general applicability of IPC on physical performance. While it may be recommended to enhance exercise capacity in males, this preconditioning technique appears less effective in females during maximal efforts.

Muscle Force Parameters

IPC affected muscle force production and the ability to resist neuromuscular fatigue differently between sexes. While male athletes increased peak and average maximal concentric force in every set of the protocol following IPC, the benefits in females were lesser (**Figures 1**, **2**). Females only displayed small improvements in peak force in the first three sets, while average force changes were trivial across all sets. Importantly, while IPC yielded acute positive responses in every male, four females out of eight experienced a decrease in performance. Another study did not report any sex difference in repeated-sprint performance, but the IPC protocol employed did not yield any positive effects in either males or females and, therefore, sex-related differences could not be truly assessed (Gibson et al., 2015). Although not studying sex differences *per se*, Gibson and colleagues (Gibson et al., 2013) reported altered 30-m running sprint times after IPC in females, while males displayed no added benefit. Taken together, these results highlight the importance of considering inter-individual responses to IPC in sports, particularly in female athletes. Considering the complex sex modulation of preconditioning mechanisms (such as mitochondrial K_{ATP} channel activation, reactive oxygen species generation, nitric oxide synthase activity, and inflammatory mediator production), as well as robust data documenting that females experience an innate protective mechanism after several forms of acute injury (for review see Pitcher et al., 2005), one could expect a sex-specific impact of remote IPC on physical performance. That said, both sexes are capable of being preconditioned (Pitcher et al., 2005). Hence, it is possible that females in the current study did not reach a sufficient threshold for preconditioning to occur. Thus, contrary to the proposition of a responder vs. non-responder pattern (Beaven et al., 2012; Gibson et al., 2013, 2015), the current data coupled with other clinical studies rather suggest that females may require a greater stimulus for effect. This remains to be elucidated by investigating the influence of varying numbers of IPC cycles and/or the number of limbs occluded at one time.

The sex-specific impact of IPC on fatigability has not been robustly assessed in the literature. The present data demonstrated clear differences between sexes; compared with males, females exhibited a lower force decrement over the five sets of maximal, isokinetic contractions. The lack of change in males is in keeping with previous studies reporting no differences between SHAM and IPC conditions in the measured fatigue index, despite higher peak and mean power outputs during the first repetitions of a series of ten 6 s cycle sprints (Patterson et al., 2015), or higher average force during maximal voluntary knee extensions after IPC (Paradis-Deschenes et al., 2016). This apparent sex

TABLE 2 | Muscle hemodynamic and oxygenation variables in IPC and SHAM conditions for males and females.

	Females			Males			Sex difference
	SHAM	IPC	% difference (ES) 90% CL	SHAM	IPC	% difference (ES) 90% CL	(ES) 90% CL
$[HHb]_{base}$ (μM)	25.3 ± 1.3	25.2 ± 1.1	−0.20%, ES −0.01, −0.35;0.33	37.5 ± 2.7	37.8 ± 2.8	0.85%, ES 0.03, −0.14;0.21	−5.39;7.92
$[THb]_{base}$ (μM)	46.1 ± 3.5	43.8 ± 3.3	−4.80%, ES −0.21, −0.50;0.08	72.2 ± 5.3	72.6 ± 5.6	0.42%, ES 0.02, −0.12;0.15	ES 0.16, −0.06;0.38
$\Delta[THb]_{rest}$ (μM)	0.1 ± 0.4	1.6 ± 0.5	1.5%, ES 1.06*, 0.13;1.99	3.6 ± 0.6	5.1 ± 0.8	1.5%, ES 0.69*, −0.17;1.55	ES 0.00, −0.80;0.79
$\Delta[THb]_{rec}$ S1 (μM)	7.3 ± 1.0	7.8 ± 0.7	0.53%, ES 0.16, −0.27;0.59	9.1 ± 1.1	10.3 ± 1.3	1.16%, ES 0.33*, −0.14;0.80	ES 0.21, −0.46;0.88
$\Delta[THb]_{rec}$ S2 (μM)	5.0 ± 0.8	5.8 ± 0.5	0.75%, ES 0.23*, −0.09;0.54	6.4 ± 0.9	6.5 ± 1.5	0.14%, ES 0.04, −0.50;0.58	ES −0.20, −0.88;0.48
$\Delta[THb]_{rec}$ S3 (μM)	3.7 ± 0.8	5.5 ± 0.8	1.77%, ES 0.54*, 0.04;1.04	6.0 ± 1.0	6.5 ± 1.4	0.57%, ES 0.16, −0.21;0.53	ES −0.40, −1.04;0.25
$\Delta[THb]_{rec}$ S4 (μM)	4.7 ± 1.1	5.2 ± 0.7	0.58%, ES 0.18, −0.24;0.59	5.3 ± 1.1	5.9 ± 1.0	0.64%, ES 0.18, −0.26;0.62	ES 0.02, −0.62;0.66
$\Delta[THb]_{rec}$ S5 (μM)	5.5 ± 0.9	5.6 ± 0.77	0.08%, ES 0.02, −0.23;0.28	3.9 ± 0.9	5.4 ± 1.1	1.54%, ES 0.44*, 0.17;0.70	ES 0.48*, 0.10;0.87
ΔReoxy ($\mu M.s^{-1}$)	0.1 ± 0.0	0.1 ± 0.02	−21.0%„ ES −0.32, −0.89;0.26	0.2 ± 0.1	0.2 ± 0.1	−4.0%„ ES −0.05, −0.38;0.028	ES 0.21, −0.29;0.70
$\Delta[HHb]_{peak}$ S1 (%AO)	59.0 ± 5.5	45.8 ± 13.3	−29.5%, ES −0.97, −2.86;0.92	69.8 ± 5.4	73.9 ± 3.9	7.3%, ES 0.26, −0.38;0.90	ES 1.35, −0.92;3.61
$\Delta[HHb]_{peak}$ S2 (%AO)	72.6 ± 7.3	57.3 ± 11.7	−10.3%, ES −0.30, −0.91;0.31	79.0 ± 5.6	83.9 ± 4.3	7.4%, ES 0.27, −0.35;0.89	ES 0.58, −0.24;1.40
$\Delta[HHb]_{peak}$ S3 (%AO)	75.0 ± 7.8	60.7 ± 11.8	−42.8%, ES −1.55, −4.11;1.01	80.4 ± 5.7	85.5 ± 3.9	7.7%, ES 0.28, −0.36;0.92	ES 2.04, −0.99;5.06
$\Delta[HHb]_{peak}$ S4 (%AO)	71.7 ± 7.3	61.4 ± 11.4	−29.1%, ES −0.95, −2.42;0.51	79.7 ± 6.8	86.3 ± 4.2	10.5%, ES 0.38, −0.24;0.99	ES 1.43, −0.32;3.17
$\Delta[HHb]_{peak}$ S5 (%AO)	68.4 ± 5.7	58.8 ± 11.5	−33.7%, ES −1.14, −2.64;0.36	82.7 ± 7.1	86.2 ± 4.2	6.4%, ES 0.23, −0.40;0.86	ES 1.52, −0.27;3.31
CMER S1	10.5 ± 1.4	5.6 ± 4.3	−4.4%, ES −0.12, −1.84;1.59	13.2 ± 2.5	11.8 ± 1.2	−2.4%, ES −0.04, −0.49;0.41	ES 0.05, −1.42;1.52
CMER S2	5.7 ± 1.3	7.2 ± 9.2	23.3%, ES 0.57, −0.57;1.72	5.6 ± 0.6	6.1 ± 0.8	8.7%, ES 0.14, −0.21;0.49	ES −0.29, −1.29;0.72
CMER S3	5.1 ± 1.1	9.9 ± 5.7	22.2%, ES 0.55, −0.46;1.56	5.1 ± 0.6	5.2 ± 0.6	3.8%, ES 0.06, −0.24;0.37	ES −0.37, −1.26;0.52
CMER S4	4.9 ± 1.0	17.3 ± 8.8	72.4%, ES 1.49, −0.42;3.41	4.7 ± 0.6	4.9 ± 0.5	5.0%, ES 0.08, −0.25;0.41	ES −1.13, −2.75;0.49
CMER S5	4.8 ± 0.9	19.3 ± 11.4	70.5%, ES 1.46, −0.30;3.23	4.6 ± 1.8	4.5 ± 0.5	−1.6%, ES −0.03, −0.36;0.30	ES −1.25, −2.75;0.25

Values are mean ± SE.

Asterisks (*) denote "clear" effect sizes (see statistics section). AO, arterial occlusion; CMER, contraction metabolic efficiency ratio; base, baseline before the IPC manoeuver; ΔReoxy, reoxygenation rate of the muscle; S, sets.

difference in response to IPC could be attributed in part to the fact that males increased their initial and total force leading to greater subsequent metabolic and ionic perturbations (Balsom et al., 1994; Glaister, 2005). However, this cannot be the only explanation, as females clearly improved their resistance to fatigue across the sets by approximately 2%. Sex differences in the availability and use of O_2 could shed some light on these differing responses to IPC.

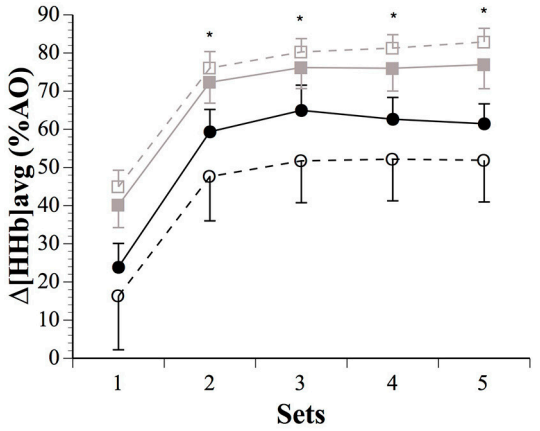

FIGURE 3 | Average changes in ($\Delta[HHb]_{avg}$), expressed as a fraction of the maximal values obtained during a transient arterial occlusion (AO) in males (SHAM: ■ IPC: □) and females (SHAM: ● IPC: ○). *Asterisks (*)* denote "clear" differences between sexes (see statistics section). Values are mean ± SE.

Muscle Hemodynamic and Oxygenation

IPC is known to increase blood flow in both ipsilateral (Kraemer et al., 2011) and contralateral limbs (Enko et al., 2011). It also up-regulates endothelial function at rest (Moro et al., 2011), after local transient ischemia (Kharbanda et al., 2001; Loukogeorgakis et al., 2005), and prevents the decline in flow-mediated dilation observed after strenuous exercise (Bailey et al., 2012a) in males. By investigating the NIRS-derived [THb] changes from pre- to post-IPC, we confirmed the acute increase in local blood volume at rest in males, and extended this hyperperfusion finding to females. In fact, these moderate to large hemodynamic changes from baseline were similar in both sexes, of a magnitude of 1.5%. Such data from female participants are very scarce in the literature, and results do vary. While Kharbanda and colleagues reported no sex difference in flow-mediated dilation response during ischaemia-reperfusion after IPC (Kharbanda et al., 2001), the same response was found to be higher in females compared with males immediately post-preconditioning (Moro et al., 2011). There is, however, stronger evidence of a sex difference in vasodilator responsiveness. Females display higher brachial artery flow-mediated dilation (Levenson et al., 2001) and forearm vasodilatory response to acetylcholine and β2-adrenergic receptor stimulation (Dietz, 1999; Kneale et al., 2000) relative to males. At first glance, these sex-based differences in intrinsic physiological responses could explain the differing impact of IPC on performance in males vs. females observed in the current study. Greater effects on endothelial function should improve contractile activity (and/or efficiency) by allowing a better O_2 supply to skeletal muscles during intense exercise. It is not clear why males had a greater increase in muscle force than females for a similar percent increase in $\Delta[THb]_{rest}$, but one could argue that males might benefit more than females from an up-regulated endothelial function since they possess a lower intrinsic vasodilator responsiveness. Nevertheless, NIRS does not offer a robust assessment of blood flow since it does not detect change in blood velocity (Delorey et al., 2003). Studies using Doppler ultrasound are warranted to investigate vasodilation and potential blood flow changes following IPC in males and females.

An augmented blood volume before exercise could facilitate O_2 delivery to active skeletal muscles. While IPC-induced changes in peak muscle O_2 extraction were trivial in males, they displayed meaningful changes in $\Delta[HHb]_{avg}$ across the sets (**Figure 3**). Males extracted more O_2 than females after IPC in sets 2–5, with clearly increased muscle force. IPC has been reported to increase systemic maximal O_2 uptake (De Groot et al., 2010), as well as local tissue deoxygenation at task failure during handgrip exercise at 45% maximal voluntary contraction (Barbosa et al., 2015), and decrease blood lactate accumulation during submaximal running exercise (Bailey et al., 2012b). IPC also accelerates muscle deoxygenation dynamics and enhances performance during whole-body cycling and sustained isometric contraction of the knee in males (Kido et al., 2015; Tanaka et al., 2016). However, despite large $\Delta[THb]_{rest}$, IPC decreased $\Delta[HHb]_{avg}$ in females in the current study. The above studies exclusively recruited males, thus the current study adds to the literature by demonstrating that IPC might not induce similar metabolic responses in female athletes. Although we did not measure blood flow *per se*, the similar $\Delta[THb]_{rest}$ after IPC in both sexes suggest that the sex difference in O_2 extraction might not be directly related to a difference in O_2 availability. Furthermore, intramuscular pressure is positively correlated with contraction intensity, and it is accepted that occlusion of muscle blood flow occurs at 50–60% maximal voluntary contraction (Wigmore et al., 2004), thereby limiting its impact on sex differences observed in O_2 metabolism when contractions are performed maximally as in the current study. In fact, the excessive intramuscular pressures of the contractions made our [THb] data collected during contractions unusable. And along this line of reasoning, it is of note that sex differences in fatigue development and performance disappear when blood flow is occluded (Maughan et al., 1986; Yoon et al., 2007). This could suggest that O_2 availability does not explain the current finding of upregulated O_2 extraction in males only. Caution is of course needed when interpreting NIRS-derived results due to methodological confounding factors such as subcutaneous fat layer thickness (although it was below the recommended emitter-receptor distance in both sexes) and possible NIRS sensor movement on the skin. Finally, muscle re-perfusion and thereby re-oxygenation occurring between maximal efforts is correlated with the recovery of muscle performance mainly via the resynthesis of phosphocreatine and by-products removal (Kime et al., 2003; Billaut and Buchheit, 2013). However, sex differences in $\Delta[THb]_{rec}$ were mostly unclear, as was the case for ΔReoxy. Based on these data, the sex-specific impact of IPC on exercise performance does not appear to be attributed to recovery processes.

There is also the possibility of a preferential impact of IPC on type II muscle fibers. A lower proportional area of type I fibers has been found in the vastus lateralis of males compared with females (Simoneau et al., 1985; Staron et al., 2000). Type II fibers display greater fractional O_2 extraction with faster kinetics and lower microvascular O_2 partial pressure (i.e., better muscle

O_2 diffusion index), despite a lower overall O_2 consumption (Mcdonough et al., 2005). Interestingly, extraneous infusion of adenosine, which is a key acting molecule released during IPC, preferentially enhances vasodilation of arterioles to type II fibers (Wunsch et al., 2000). Therefore, due to the greater O_2 extraction of type II fibers when highly perfused (Wilson et al., 1977), it has been suggested that these fibers might benefit more from an increase in blood perfusion than the more aerobic type I fibers (Faiss et al., 2013; Paradis-Deschenes et al., 2016), which could explain the higher $\Delta[HHb]_{avg}$ in strength-trained males in the present study.

In conclusion, this applied study demonstrated that strength-trained males might benefit more clearly from IPC than their female counterparts during repeated, maximal contractions. This strengthens clinical observations that sex may be a confounder in the response to this stimulus. Despite a similar increase in blood volume (↑[THb]) in both sexes immediately after IPC, and thus presumably similar increase in O_2 availability, males displayed greater peripheral O_2 extraction (↑Δ[HHb]). This ergogenic effect therefore appears to be mediated in part via an upregulation of oxidative function in males, possibly within type II muscle fibers.

AUTHOR CONTRIBUTIONS

PPD, DRJ, and FB conceptualized and designed the research project; PPD acquired the data and conducted the statistical analysis; PPD interpreted results with assistance from DRJ and FB; PPD wrote the manuscript with revisions from DRJ and FB. All authors reviewed and agreed upon the final manuscript.

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OPINION
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Walking in Hypoxia: An Efficient Treatment to Lessen Mechanical Constraints and Improve Health in Obese Individuals?

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Obesity is defined as a body mass index >30 kg/m^2 and is a major health burden in many parts of the world (Finucane et al., 2011). The incidence of worldwide obesity is escalating at an increasing rate and has more than doubled since 1980. To tackle body fat accumulation and its clinical complications (e.g., diabetes, hypertension, heart disease), aggressive prevention strategies that are mainly based on dieting and lifestyle change have been implemented (Bray et al., 2016). Regular physical exercise such as walking is also generally recommended to increase energy expenditure (Donnelly et al., 2009). Despite an increasing and on-going body of research on cardio-metabolic disorders associated with the obese phenotype (Atkinson, 2014), many questions remain unanswered. Adherence to prescribed or spontaneous exercise remains low in obese patients (Dalle Grave et al., 2011), which raises specific questions on the effectiveness (Malhotra et al., 2015), as well as the objective (Browning and Kram, 2007) and subjective (Annesi, 2000) difficulties of exercising in these patients.

Due to discomfort, it remains unclear how excessive adipose tissue contributes to lower levels of physical activity as well as lower mobility and functional performance. Furthermore, obesity may increase joint stresses during simple locomotion tasks such as walking, which can lead to aberrant mechanics, reduced range of motion in the joints and eventually musculoskeletal pathologies (e.g., lower-extremity osteoarthritis, rheumatoid arthritis and/or low back pain) (Wearing et al., 2006; Browning, 2012; Sheehan and Gormley, 2012). For many obese patients, the reality of musculoskeletal disorders such as knee osteoarthritis may outweigh the eventual benefits of physical activity and weight loss. Altogether, non-compliance by obese patients to current exercise prescriptions and recommendations (Donnelly et al., 2009) suggests that existing exercise regimes do not necessarily meet the needs of this population. Alternative strategies are therefore required and their effectiveness must be clinically validated.

Hypoxic exposure results in any inspired pressure of oxygen under a normoxic value of 150 mmHg (Conkin and Wessel, 2008), while exercising is considered a new therapeutic strategy (Kayser and Verges, 2013; Millet et al., 2016). Until now, the combination of hypoxia and exercise stressors had mainly been investigated in normal weight patients (body mass index <25 kg/m^2) or lean individuals. The focus of the very few existing studies that included obese patients was on metabolic and body composition changes and not on walking or biomechanical improvement (Netzer et al., 2008; Haufe et al., 2010; Wiesner et al., 2010; Gatterer et al., 2015). Therefore, while cardio-metabolic adaptation was the main target of available studies on obesity (Atkinson, 2014; Verges et al., 2015), biomechanical investigations where hypoxia would be manipulated are also necessary. This is potentially useful to advance basic science as well as to develop effective physical activity recommendations to achieve energy expenditure goals while reducing the risk of musculoskeletal injury in obese patients (**Figure 1**).

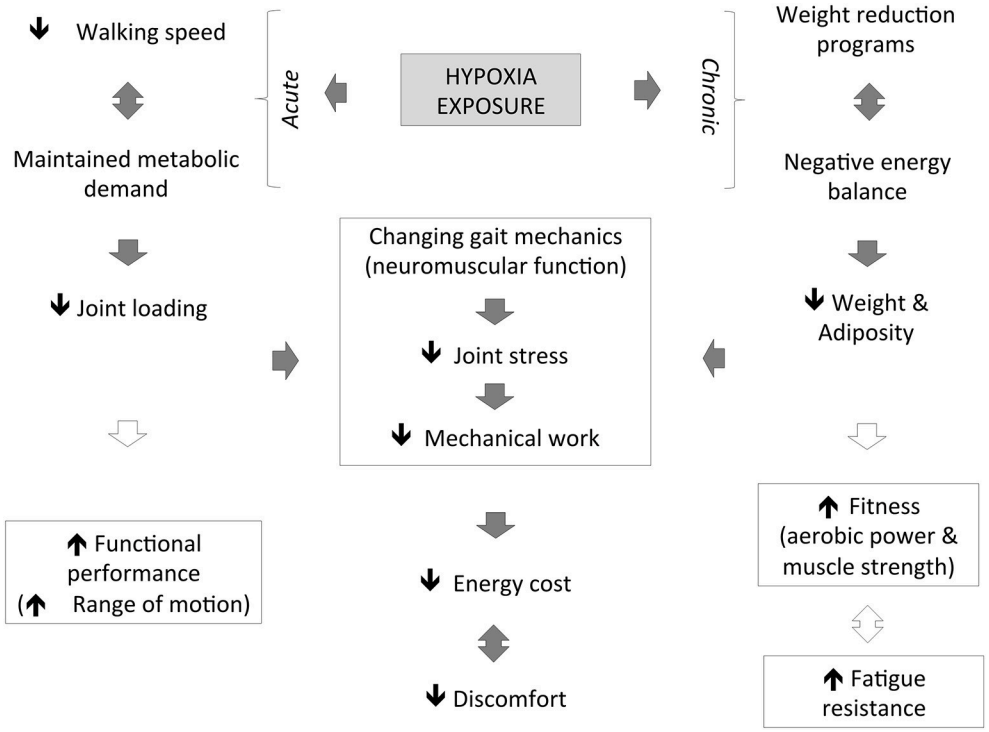

FIGURE 1 | Illustration of the hypothetical effects of hypoxia exposure on neuro-mechanical constraints associated with walking in obese patients

LOCOMOTION MECHANICS IN OBESE PATIENTS

Compared to lean individuals, preferred walking speeds have been consistently reported to be slower (i.e., typically 10–15%) in obese patients, with walking speeds that also appear inversely related to body mass index (Browning and Kram, 2005; Malatesta et al., 2009). This is presumably because of disproportionately heavier limbs, reduced relative muscle strength and a greater need to maintain balance during ambulation (Sheehan and Gormley, 2012). Obese patients tend to walk with a longer period of double support (i.e., both feet on the ground) and they swing their legs more rapidly (e.g., shorter swing time) with more lateral circumduction (e.g., wider step width) as well as abnormal (e.g., altered distribution) lower-extremity joint loads, absolute ground reaction forces (e.g., excessive peak forces and higher loading rates) and forefoot pressures when compared with non-obese individuals walking at identical speeds (Devita and Hortobágyi, 2003; Wearing et al., 2006; Browning, 2012). To date, there is no clear consensus on the precise nature of mechanical "mal-adaptations" associated with obesity (Sheehan and Gormley, 2012). Hence, whether a more erect sagittal plane posture and greater knee adduction, hip abduction and foot eversion are seen in obese vs. lean individuals remains controversial. As obese patients typically display a large variability in their mechanical stride parameters, it is difficult to identify common characteristics of walking patterns in this population (Wearing et al., 2006). Despite their greater body mass, absolute joint torques at the hip and knee are relatively similar to those of non-obese individuals, while ankle joint torques are approximately twice as large (Wearing et al., 2006). These biomechanical differences require that physical therapists and clinicians should make specific recommendations when prescribing physical exercise to obese patients.

WEIGHT-PERTURBATION INTERVENTIONS

Increasing the level of physical activity is likely a crucial intervention for an efficient prevention and treatment of obesity (Donnelly et al., 2009). The importance of reducing fat-mass accumulation in obese patients is indisputable; but surprisingly, only a few studies addressing the positive effects of weight loss on changes in walking biomechanics exist. Notwithstanding, massive weight loss produces dramatic reductions in knee forces during walking but when patients walk faster, these favorable reductions become substantially attenuated (Devita et al., 2016).

Because of various interventions (e.g., exercise training, Peyrot et al., 2010) or bariatric surgery (Hortobágyi et al., 2011), mechanical alterations (e.g., lower knee joint loads) and reductions in musculoskeletal pain may minimize the net energy cost. The combination of slower walking speeds and moderate inclines (0.75 m/s, 6°) is another intervention that is metabolically similar to a "normal" level of walking (1.50 m/s, 0°), yet it is associated with reduced net-muscle moments and loading rates (Ehlen et al., 2011). Furthermore, a faster stride frequency when walking speed is held constant (an example

of "gait retraining") likely increases (∼5%) energy expenditure without negatively affecting walking mechanics (Russell et al., 2010). Finally, a 10% weight loss through dietary intervention reduced knee compression by 200 N and reduced pain and disability in obese adults with knee osteoarthritis (Messier et al., 2013). Reducing potentially harmful forces in the knee during walking would improve locomotion in obese individuals.

In clinical rehabilitation settings, reducing musculoskeletal loading using lower body-pressure treadmills has an unprecedented popularity. For example, a 2016 meta-analysis indicated that peak and active vertical ground-reaction forces were consistently reduced in artificially weight-reduced healthy individuals; unweighting also provided some horizontal assistance and altered regional loading within the foot toward a forefoot strike (Farina et al., 2017). A potential drawback is that decreased speed with body-weight support will also reduce energy expenditure (e.g., oxygen uptake and heart rate readings) and muscle activation (i.e., with different responses between both stabilizer and propulsive muscles), likely minimizing any stimulus training effect. Use of a lower-body positive-pressure treadmill requires wearing tight neoprene shorts that are then attached to the treadmill and probably limits the range of motion of certain lower extremity joints and balance. One also cannot rule out that an improper arm swing leads to modified gait mechanics.

HYPOXIC EXPOSURE

Browning and Kram (2007) calculated that obese patients would need to walk at approximately 1.1 m/s (i.e., close to their preferred walking speed) to have a biomechanically equivalent joint load as lean individuals walking at 1.4 m/s. Consequently, obese patients would have to walk faster than their preferred walking speed to increase exercise intensity and to match the current physical activity guidelines (Donnelly et al., 2009). However, lower-extremity joint loads and the associated risk of musculoskeletal disorders likely increase with walking speed (Browning and Kram, 2007). In this context, acute hypoxia exposure may become advantageous as the mechanical load during physical exercise under hypoxic vs. normoxic conditions would be significantly reduced to achieve the same metabolic effect. In short, hypoxia enabled obese patients to achieve a higher metabolic demand, while a lower walking speed was also likely more protective of the muscles/joints in obese patients with orthopedic comorbidities (Wiesner et al., 2010).

To limit the negative effect of increased level walking speed on mechanical constraints, it is important to determine if walking in an O_2-deprived environment at a slower speed represents an effective strategy to reduce the load across the lower extremity joints, while providing adequate (i.e., similar to faster walking speeds near sea level) physiological stimulus for weight management. In healthy older community dwellers, changes of time-based gait parameters (e.g., slower cadence, longer stride time, and larger temporal gait variability) from the beginning to the end of a 40-min treadmill walk occurred, but these fatigue-related effects were similar to a 2,600-m simulated altitude or those near sea-level (Drum et al., 2016). Conversely and while exposed to hypoxia, obese subjects may re-organize their neuromuscular function to produce gait patterns that result in different knee joint loading. Based on the findings of studies conducted in healthy populations with lower-body positive-pressure treadmills, we postulated that most muscles that are involved in body-support would demonstrate a reduction in muscle activity as mechanical constraints would decrease with acute hypoxia exposure. Changes in muscle activity are not directly proportional to the changes in "unweighting" for all muscles, and vary widely across muscles [i.e., activation is not decreased in certain muscles (e.g., tibialis anterior, rectus femoris) until considerable unweighting occurs, Sainton et al., 2015, while others (e.g., hip adductor; Hunter et al., 2014) will barely change] and include the severity of hypoxic stress. This type of exercise intervention may be particularly useful to develop familiarity and compliance with regular physical exercise, especially when ambulation becomes less painful (Hootman et al., 2002; Ekkekakis and Lind, 2006).

Hypoxic conditioning consisting of chronic hypoxic exposure or sessions of intermittent exposure to moderate hypoxia repeated over several weeks may induce hematological, vascular, metabolic and neurological effects (Verges et al., 2015). This is presently considered as a promising therapeutic modality for several pathological states (e.g., heart failure, stroke, spinal cord injury patients) including obesity (Urdampilleta et al., 2012; Kayser and Verges, 2013; Millet et al., 2016). Continuous hypoxic training (low intensity endurance exercise for 90 min at 60% of the heart rate at maximum aerobic capacity, three times per week for 8 weeks; inspired fraction of $O_2 = 15\%$) in overweight subjects (body mass index >27 kg/m^2) leads to larger (+1.14 vs. 0.03 kg) weight loss than similar training in normoxic environments (Netzer et al., 2008). The effectiveness of such a low-intensity intervention remains questionable over a longer period of 8 months since Gatterer et al. (2015) did not report higher reductions in body weight between hypoxic and normoxic interventions. However, the biomechanical consequences of chronic hypoxic interventions on the walking pattern of obese patients are simply unknown.

To date, epidemiological reports associate the moderate altitude of residence to lower obesity prevalence without clear underlying mechanisms (Voss et al., 2014; Woolcott et al., 2016). Recently, it was reported that O_2 variations in organic systems may lead to considerable (3%) weight loss (yet of undefined composition) and improve metabolic and cardiorespiratory health (Netzer et al., 2008; Kayser and Verges, 2013; Kong et al., 2014). This leads to the suggestion that sustained hypoxia may be of benefit to weight management programs in obese patients (Millet et al., 2016). For prolonged exposure, the so-called "altitude anorexia" mechanisms that lead to a reduced appetite in altitude cannot be ruled out (Tschop and Morrison, 2001). The explanation for this phenomenon remains unclear but has been related to a modification in appetite regulation hormones (Shukla et al., 2005).

PERSPECTIVES

Acute Hypoxia

- On a lower-body positive-pressure treadmill, faster speeds are required to reach similar exercise intensities than on a normal treadmill (Farina et al., 2017). Reportedly, faster walking and running speeds (6–16 km/h) rather than increases in percent body weight (50–100%) cause a greater maximum plantar force on a lower-body positive-pressure treadmill (Thomson et al., 2017). These observations are limited in scope to healthy male runners. When combined, acute hypoxia exposure (normobaric hypoxia) to artificially increase the metabolic load and exercise on body positive-pressure or aquatic treadmills to decrease the mechanical strain might be clinically relevant. Deeper investigations of obese patients walking at slower speeds (e.g., ranging 0.5–3.5 km/h) with and without hypoxic exposure are required and are likely to alter the relationship between speed and muscle unweighting on mechanical strain as reported in similar studies (Thomson et al., 2017). While exercising at simulated altitudes ranging from 2,500 to 3,500 m is commonly implemented (Haufe et al., 2010; Kong et al., 2014; Gatterer et al., 2015), the optimal degree of hypoxic severity (i.e., maximize physiological adaptations with limited negative consequences) is unknown (e.g., Urdampilleta et al., 2012 for intermittent hypoxic exercise recommendations), and requires further study. However, it is likely that due to the maladaptive side effects associated with hypoxia (i.e., sleep apnea, intermittent hypoxemia), obese patients would not tolerate a high-altitude (Dempsey and Morgan, 2015). For safety reasons, we speculate that hypoxic training could be performed at a simulated altitude lower than 3,500 m.
- Thus far, the available obesity-related studies investigated gait mechanics prior to any signs of fatigue. Perturbations in muscle force generation could reduce the ability of fatigued muscle groups to attenuate to ground reaction impact forces, thereby exaggerating gait abnormalities, and likely increasing the fall risks during locomotor tasks (Himes and Reynolds, 2012). To date, there is a dearth of information pertaining to the changes in gait and balance control over time as obese patients start to fatigue. It is also unknown whether there is a cumulative effect of hypoxic exposure and exercise-induced fatigue on locomotion mechanics that may increase fall risks in obese individuals who are exercising for a prolonged time at moderate altitude.
- Walking includes an inherent fall risk based only on the mere exposure to gradients and surface variations (e.g., stair climbing, rocky paths) that would influence the load applied to the weight-bearing joints of obese individuals. Surface electromyography (EMG) recording during various locomotive tasks would elucidate muscle activation strategies used by obese patients to cope with these situations, including whether hypoxia exposure modifies neuromuscular responses. While the surface EMG may be feasible in severely obese individuals (Minetto et al., 2013), decomposition of surface EMG signals into time-frequency components, wavelet components and degrees-of-freedom force functions may provide further insight into the contributions from the neuromuscular system (Hamid Nawab et al., 2010).

Chronic Hypoxia

- Weight loss is an important method for the treatment of obesity and its associated comorbidities. It is possible to measure the net energy cost of level-walking in obese patients by investigating the effect of decreased body mass on gait pattern and external mechanical work (i.e., simple inverted pendulum modeling to approximate the energy required to raise and accelerate the center of mass; Malatesta et al., 2013). To date, moderate-intensity continuous training is the type of physical activity most frequently recommended to obese patients (Donnelly et al., 2009). That said, growing evidence suggests that high-intensity interval training is a time-efficient approach in this population (Kong et al., 2016). However, this training was rated as less pleasant and less enjoyable than an isocaloric session of moderate-intensity continuous exercise (Decker and Ekkekakis, 2017). Future studies are warranted that compare the effects of various exercise modalities/intensities in addition to hypoxia exposure on the changes in gait pattern.
- Hypoxic training embraces different methods as "live high–train high," "live high–train low," or "live high–train low" interspersed with hypoxic training for additional sessions ("live high–train low and high") that can be conducted in normobaric or hypobaric (natural) conditions with the use of artificial devices or by ascending to elevated terrestrial environments (Millet et al., 2013). To our knowledge, there are no studies that compared the influence of these different altitude-training methods on the weight loss and gait mechanics in obese patients. Consequently, how different physiological adaptations and different degrees of body mass loss associated with various hypoxic training methods would specifically affect gait pattern in obese patients remains undetermined.
- Walking and hiking for hours at low-to-moderate intensity in mountainous areas is a popular and safe outdoor activity, even for obese patients (Neumayr et al., 2014), and should therefore be recommended to increase physical activity in this population. The potential identification of a significant difference between simulated (i.e., normobaric hypoxia) and terrestrial (i.e., hypobaric hypoxia) altitudes is also clinically relevant. Interestingly, Degache et al. (2012) reported that real altitude superiorly decreased postural stability. Investigations that compare normobaric to hypobaric hypoxia in obese patients are currently lacking.

CONCLUSION

Obese patients should be enthusiastically encouraged to engage in regular physical activity for the improvement of cardio-metabolic health, with exercises selected that minimize the joint load and pain as much as possible. Walking at slower speeds under hypoxic conditions would reduce joint loading (and the risk of musculoskeletal injury/pathology), while

ensuring an adequate exercise stimulus for weight management. Alternatively, hypoxic conditioning may be an appropriate form of exercise training for obese patients as it can lead to effective weight loss due to a negative energy balance. These acute and chronic hypoxic-related interventions would contribute to the support of an appropriate and individualized prescription for obese patients to reduce the biomechanical load involved in walking and eventually improve training adherence (**Figure 1**).

AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

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Endurance Training in Normobaric Hypoxia Imposes Less Physical Stress for Geriatric Rehabilitation

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Rationale: Evidence suggests that training in hypoxia can be very effective even while requiring less physical effort. We therefore aimed to measure the effect of endurance training under hypoxic conditions on pulmonary and cardiovascular parameters in an elderly population undergoing inpatient rehabilitation.

Methods: Forty patients over age 65 years with multiple co-morbid conditions were recruited during a 3-week stay in a geriatric rehabilitation center. Using a randomized, single-blinded, placebo-controlled design, patients were assigned to a hypoxic (HG) or normoxic (NG) group. HG patients completed seven training sessions of 30 min duration on a treadmill in a normobaric chamber with inspired oxygen fraction (FiO_2) of 15.27%, with 10–30 min active training. Training was conducted with target heart rate at 80% of peak oxygen consumption (VO_2-peak). NG group performed similar training in sham hypoxia (room air or $FiO_2 = 20.94\%$). At pre- and post-test completion, measures included: (1) cycle ergometry with ECG monitoring and measurement of VO_2-peak, and (2) echocardiography for ejection fraction.

Results: The physical effort required of patients to reach target heart rate was reduced significantly (-28%, $p = 0.043$) in the HG compared to NG. Cardiopulmonary parameters showed no differences between groups.

Conclusion: Endurance training at 3,000 meters elevation imposes less stress on the locomotor systems while resulting in a similar physiological strain (i.e., heart rate). Hypoxic training holds promise for successful geriatric rehabilitation by being more accommodating to physical limitations in geriatric patients.

Trial registration: Registration at DRKS. (Approval No. 359/12, Trial No. DRKS00005241).

Keywords: hypoxia, training with geriatrics, low external load, training, geriatrics

INTRODUCTION

With the aging population, rehabilitative training for the elderly is gaining humanitarian and economic importance (Poterba, 2016). A major goal is to keep the elderly as fit and active as possible to prevent immobility and hospitalization (Jamour et al., 2014). This effort has the potential to improve quality of life and decrease burdens on the medical system. Despite intensive investigations into methods for training and therapy, there is a paucity of data and evidence-based guidelines for endurance training in the elderly ≥75 years (Meusel, 2000). However, endurance training does show positive results (Meusel, 2000; Oster et al., 2005; Fiogbe et al., 2017; Pandey et al., 2017). For common diseases such as coronary heart disease, stroke, cancer and diabetes, endurance training has proven to be effective in younger individuals <75 years (Buchner et al., 1997; Cornelissen and Fagard, 2005; Ventura-Clapier et al., 2007). Since those diseases have a high prevalence among geriatric patients, the question is raised regarding the benefit of such endurance training in the elderly. Restrictions in endurance capacity have a direct impact on immobility, muscle atrophy and frailty, all of which relate to activities of daily living (Avila-Funes et al., 2011). Recent studies in the field of endurance training suggest a greater effect of training at higher intensities (Hayashi et al., 2005; Swain and Franklin, 2006; Pokan et al., 2009). Higher intensities of training are especially hard to reach with geriatric patients because of physical and medical limitations. High-intensity training is even harder in rehabilitation settings such as after surgery. At times tolerance of active training may be reduced to 10–20 min per day. For endurance training to provoke adaptive responses in the cardiopulmonary systems in such a short time period, it may be necessary to conduct the training at 60–80% of peak oxygen (O_2) consumption (VO_2-peak) (Hayashi et al., 2005; Swain and Franklin, 2006; Pokan et al., 2009). Attaining this intensity level of training in the elderly may not be possible without substantial risk (Oster et al., 2005).

Positive effects of hypoxic training have been shown in prior investigations notably in obese subjects (Piehl Aulin et al., 1998; Netzer et al., 2008; Schipfer et al., 2008; Haufe, 2010; Gatterer et al., 2014). Initially, endurance performance in hypoxia is reduced, but even when applying a lower external load, such hypoxic training can result in cardiovascular and metabolic responses that are similar to high-intensity training in normoxia (Kong et al., 2017).

These observations suggest that hypoxic training may be useful to increase training intensity for geriatric patients while maintaining a low impact on locomotor systems. Hypoxic training may also have positive effects on ejection fraction (EF), heart rate (HR) to power ratio, O_2 consumption (VO_2), and revascularization of the myocardium (Knuth, 2008). In this study our aim was to measure the effect of endurance training under hypoxic conditions on pulmonary and cardiovascular parameters in an elderly population undergoing inpatient rehabilitation. We hypothesized that endurance training in normobaric hypoxia may induce a lower physical stress for a similar physiological strain compared to similar training in normoxia. As such, this may safely support targeted endurance training while respecting physical restriction in geriatric patients.

METHODS

Subjects were recruited from the geriatric rehabilitation hospital (Fachklinik Ghersburg für Geriatrische Rehabilitation, Bad Aibling, Germany) attached to the study center. Inclusion criteria for participation were: (1) age > 65 years, (2) cognitive ability to give informed consent and to participate in the study, (3) sufficient peripheral blood flow for pulse oximetry, (4) heart failure symptoms no worse than NYHA Class III, and (5) dosages of cardiovascular-relevant medications (ß-blockers, ACE inhibitors, etc.) that would allow at least a 10% increase of HR to physical stress. An exclusion criterion was any orthopedic condition that precluded performance of a cycle ergometry test.

Of the 40 patients recruited, 35 (87.5%) were able to complete the trial. Reasons for dropout were unwillingness to continue ($n = 4$) and termination for medical reasons ($n = 1$).

Descriptive means for the normoxic group (NG, $n = 16$) were: age 82.0 (±7.8) years, sex 11 women/5 men, and BMI 25.8 (±6.2) kg/m^2. Descriptive means for the hypoxic group (HG, $n = 19$) were: age 80.2 (±7.2) years, sex 12 women/7 men, and BMI 25.2 (±5.3) kg/m^2.

Exercise Testing

At pre-test, anthropometric data were collected and echocardiography was performed. Cycle ergometry with ECG monitoring was conducted using a standard protocol for heavily impaired patients (Pokan et al., 2009). After 3 min of rest and 3 min of reference pedaling at 15 watts, the test started at 27 watts and was increased by 7 watts each minute until subjective exhaustion (Pokan et al., 2009). Criteria that would trigger interruption of the test were: (1) HR higher than maximal predicted, calculated using the formula 220 bpm—age, (2) systolic blood pressure (BP) higher than 220 mmHg, (3) any ST-segment changes, (4) ventricular extrasystoles, or (5) angina pectoris. The testing was also interrupted upon attaining subjective exhaustion equivalent to 18 on the BORG-Scale (Borg, 1982). After pre-test, patients were randomly assigned in equal numbers to the HG or the NG.

Training Phase

The subsequent target HR for training was set as 80% of VO_2-peak (maximum O_2 consumption achieved during the pre-test ergometry test) with 5 bpm added to account for the conversion from cycle to treadmill training (Kroidl et al., 2015). The more standard conversion factor of 10 bpm was not used because patients most commonly train with their arms resting on the treadmill handrails. The main parameters that were used to assess training effects were EF by echocardiogram, peak HR, O_2 saturation, VO_2-peak, and peak O_2/HR. Patients were asked to perform 7 training sessions during their 3-week stay in the geriatric rehabilitation center. The normobaric hypoxic chamber was set to contain either hypoxic air equivalent to an altitude of 3000 meters ($F_iO_2 = 15.27\%$) or sham hypoxia (room air at $F_iO_2 = 20.94\%$). Normobaric hypoxia was produced using

a device called "Low Oxygen Systems" (Berlin-Buch, Germany) which provided a controlled mixture of fresh air and nitrogen to keep O_2 and carbon dioxide (CO_2) levels constant. This allowed patients to exercise without wearing a mask and to concentrate fully on the intervention. Patients were blinded to the different conditions though the investigators were not (single-blinded). Each of the 7 training sessions in the chamber included a stay of at least 30 min with a minimum of 10 min and a maximum of 30 min of active training on a treadmill. The treadmill (h/p/cosmos, Traunstein, Germany) offered forearm supports for more impaired patients and a safety belt to stop the movement in an emergency. The therapists tried to reach the target HR considering the physical and medical impairments of each patient and taking care not to exceed the target HR. BP (Omron M400 IT) was measured before each training session, while HR (Polar Heart Rate Sensor T31) and O_2 saturation (NONIN Go 2) were monitored continuously during training.

The intervention protocol was designed to be practicable and achievable. The prescription of seven sessions of at least 10 min of endurance training over a 3-week period corresponded to common practice in geriatric rehabilitation hospitals. Both HG and NG subjects also participated in the other prescribed rehabilitation programs in the hospital, consisting of one 30-min individual physical therapy session and another 30-min group physical therapy session (e.g., strength, gait, balance training) per day. The mean time elapsed between training sessions as well as pre- and post-tests was 1.56 days in HG and 1.57 days in NG. At the conclusion of the 7 sessions of endurance training, outcome measurements were repeated as they had been performed at pre-test.

STATISTICS

Evidence from published research suggested that for the main outcome parameter of VO_2-peak, we would detect a mean difference of 0.23 l/min (SD $\pm$ 0.36 l/min) between intervention group and control group (Vaitkevicius et al., 2002; Vogt et al., 2002). To reach a significance level set a priori at $p < 0.05$ and a power of 80%, it would be necessary to enroll 17 subjects in each arm of the study. To account for possible dropouts, the study enrollment was set at 40 subjects in total. Data are presented as means $\pm$ standard deviation (SD). For all analyses a two-way ANOVA for repeated measures was performed. To identify the magnitude of statistical difference between the groups in each training session (Tr1-Tr7) a student *t*-Test was used. Data analyses were performed with the SPSS statistical software package (PASW Statistics for Windows version 21.0, SPSS Inc., Chicago, IL, USA). Effect size, using ANOVA, was calculated as partial eta-squared (η^2). $\eta^2 < 0.06$ accounts for a small effect, $0.06 < \eta^2 > 0.14$ accounts for a medium effect and $\eta^2 > 0.14$ accounts for a big effect.

STUDY APPROVAL

The study protocol was reviewed and approved by the institutional review board of the University of Ulm on the January 19, 2015 and was registered as a clinical trial with the German Clinical Trials Register (DRKS). (Approval no. 359/12, Trial no. DRKS00005241) Participants gave their written informed consent prior to inclusion in the study.

RESULTS

Thirty five patients (23 women) were able to finish the trial. Among these patients the most common diagnoses were coronary heart disease, diabetes types I and II, hypertension, chronic obstructive pulmonary disease, peripheral arterial occlusive disease, hip replacement, fractures, congestive heart failure, osteoporosis, stroke and myocardial infarction. The majority of patients were prescribed cardiovascular-relevant medications as well as diuretics, anticoagulant medication, cholesterol and blood sugar lowering drugs. During the study period, slight changes in medication were made by the hospital's physicians but these medication changes occurred in both groups equally.

The endurance training was well tolerated and there were no complaints regarding the intervention. For some patients, transfer to the cycle ergometer for ergospirometry was hard to accomplish. The gas analyzer mask was reported to be uncomfortable but tolerable for the short testing period.

Outcome Measurements before and after the Intervention

Statistical analysis showed no significant differences between groups. From pre- to post-test, there were no changes in anthropometric parameters (**Table 1**). There was also no change in the measured cardiologic parameters. Mean EF for the HG showed a non-significant decrease compared to the NG. Although not significant, peak VO_2 was higher in post-test in both groups. The peak O_2 /-HR was higher in HG but this difference was not statistically significant. However, the maximum performance increased significantly in both groups from pre- to post-test ($p = 0.004$) (**Table 1**). There were no sex differences regarding the effect of the intervention. Overall, both groups showed similar responses from pre- to post-test though the training intensity (% of peak VO_2) was significantly lower in HG ($p = 0.012$) (**Table 2**).

Intervention

Aiming for a mean training intensity at 80% of VO_2-peak the NG had to be pushed harder and only reached an average intensity of 74%. The HG had to be restrained to prevent them from exceeding their target HR resulting in a mean training intensity of 80%.(**Figure 1**). All intensity related parameters showed a significant reduction in training effort for HG as displayed in **Table 2**. Resting BP in both normoxic and hypoxic environments did not change over the time course of the seven training sessions (**Figure 2**).

DISCUSSION

This is the first study of endurance training in normobaric hypoxia with exclusively geriatric patients. The present study

TABLE 1 | Anthropometrical and physiological values at baseline (pre-) and after (post-) the endurance training for the hypoxic group (HG) and normoxic group (NG).

Item	Hypoxic group (HG)		Normoxic group (NG)		Condition	Time	Interaction
	Pre-	Post-	Pre-	Post-	*P*	*p* (η^2)	*p*
Weight (kg)	67.74 (±18.92)	68.21 (±19.34)	68.38 (±16.49)	67.78 (±17.75)	0.56	0.608	0.254
BMI (kg/m^2)	25.23 (±5.26)	25.41 (±5.39)	25.83 (±5.71)	25.64 (±6.43)	0.457	0.827	0.287
EF (%)	42.83 (±9.28)	39.87 (±9.31)	46.61 (±15.70)	44.2 (±9.46)	0.493	0.169	0.931
Peak Power (W)	41.18 (±13.53)	46.19 (±14.43)	43.38 (±10.41)	46.24 (±11.41)	0.737	0.004** (0.43)	0.289
Peak HR (bpm)	120.05 (±30.12)	120.37 (±24.48)	116.56 (±22.92)	120.31 (±20.41)	0.296	0.118	0.459
Peak VO_2 (ml/min)	929.68 (±325.6)	1003.37 (±342.02)	938.13 (±218.01)	969 (±296.74)	0.858	0.375	0.703
Peak O_2/HR (ml)	8.86 (±2.83)	9.4 (±3.14)	8.61 (±2.54)	8.66 (±2.59)	0.655	0.544	0.574

Values are means ± SD.
*BMI, body mass index; EF, ejection fraction of the heart; peak HR, peak heart rate; peak VO_2, peak oxygen consumption; peak O_2/HR, peak fraction of oxygen to heart rate; **$p \leq 0.025$ levels of significance effect size is displayed as η^2.*

TABLE 2 | Physiological and training-intensity related parameters during the intervention for the hypoxic group (HG) and normoxic group (NG).

Item	Hypoxic group (HG)			Normoxic group (NG)			Cond. *p* (η^2)	Time *p* (η^2)	Int. *p* (η^2)
	Tr1	Tr7	Δ Tr1-7 (%)	Tr1	Tr7	Δ Tr1-7 (%)			
Watt (W)	18.53 (±6.54)	22.84 (±13.52)	23.26	23.00 (±11.51)	34.75 (±14.90)	51.09	0.058	<0.001*** (0.56)	0.005*** (0.18)
Watt/kg (W)	0.27 (±0.07)	0.32 (±0.15)	18.52	0.33 (±0.11)	0.50 (±0.13)	51.52	0.012 (0.35)	<0.001*** (0.53)	0.001*** (0.21)
Training intensity (%)	79.45 (±7.96)	80.67 (±5.80)	1.54	68.12 (±9.03)	74.43 (±8.33)	9.26	0.012** (0.35)	0.059	0.739
BP systolic (mmHg)	140.47 (±22.78)	140.32 (±23.32)	−0.11	148.44 (±17.95)	141.88 (±19.94)	−4.42	0.350	0.163	0.605
BP diastolic (mmHg)	77.32 (±19.27)	76.68 (±10.24)	−0.83	82.06 (±8.21)	75.75 (±11.83)	−7.69	0.609	0.616	0.465
Velocity (km/h)	1.37 (±0.29)	1.37 (±0.36)	±0.00	1.44 (±0.29)	1.71 (±0.23)	18.75	0.070	<0.001*** (0.32)	0.001*** (0.21)
Grade (%)	0.12 (±0.46)	1.65 (±2.21)	1.275	1.13 (±1.75)	3.88 (±2.42)	243.36	0.005** (0.42)	<0.001*** (0.46)	0.066
Walking distance (m)	263.16 (±99.12)	381.23 (±251.04)	44.87	285.63 (±105.42)	505.42 (±178.25)	76.95	0.358	<0.001*** (0.63)	0.492
SpO_2 (%)	88.28 (±9.00)	90.11 (±11.47)	2.07	94.42 (±2.98)	95.15 (±2.33)	0.77	<0.001*** (0.72)	0.027* (0.14)	0.776

Values are means from Tr1 and Tr7 ± SD.
*Training intensity, % of peak heart rate; BP, blood pressure; SpO_2, oxygen saturation, *$p \leq 0.05$, **$p \leq 0.025$, ***$p \leq 0.001$ levels of significance, effect size is displayed as η^2.*

demonstrates that endurance training performed in hypoxia in geriatric patients requires less physical effort to produce an effective and comparable physiological strain (i.e., higher HR) than with similar training in normoxia. Of particular relevance in elderly, the significant reduction in mean power output by more than 25% during such hypoxic endurance training offers a gentler rehabilitation process for specific orthopedic limitations.

While endurance training could also be performed using an arm ergometer or similar ergometers depending on the patient's injury, it is noteworthy that the main goals of most geriatric patients are to get back on their feet and avoid future falls (Buchholz and Kohlmann, 2013). Combining gait training and endurance training during a stay in a rehabilitation center may improve therapy even while taking into consideration the reduced tolerance of physical training experienced by most geriatric patients. In view of this lowered tolerance for physical training, hypoxic training—which appears to be well tolerated, without provoking any complications—provides a new opportunity to enhance cardiac capacity while focusing on classical therapeutic goals (e.g., improvement in gait performance and postural stabilization) in geriatrics.

With the exception of peak power output, both groups reacted with the same non-significant physiological changes: a slightly increased VO_2-peak, a decrease in peak O_2/-HR and no change in EF. Evidence from related studies suggest that hypoxic exposure normalizes hypertension, improves myocardial angiogenesis or ventricular remodeling and decreases the risk for ischemic heart disease (Millet et al., 2016). Hypoxia also seems to positively affect hematological parameters as well as cognitive functioning (Schega et al., 2016). The outcomes of the present study are in line with these findings. Nevertheless, the lack of significant changes in cardiac parameters suggest that the training stimulus

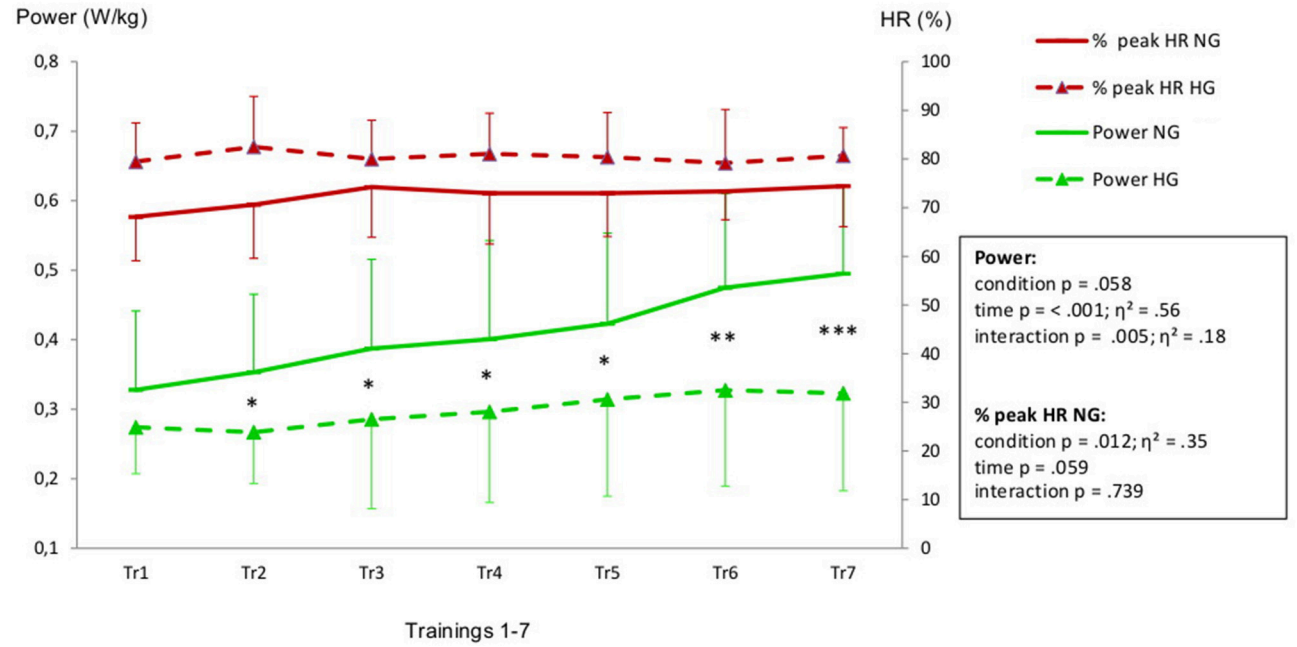

FIGURE 1 | Heart rate (expressed in % of HR peak) and power values (W/kg) recorded during the training intervention (Tr1-Tr7). Legend: $*p \leq 0.05$, $**p \leq 0.025$, $***p \leq 0.001$ levels of significant differences between HG and NG in Tr1 to Tr7. Effect size is displayed as η^2.

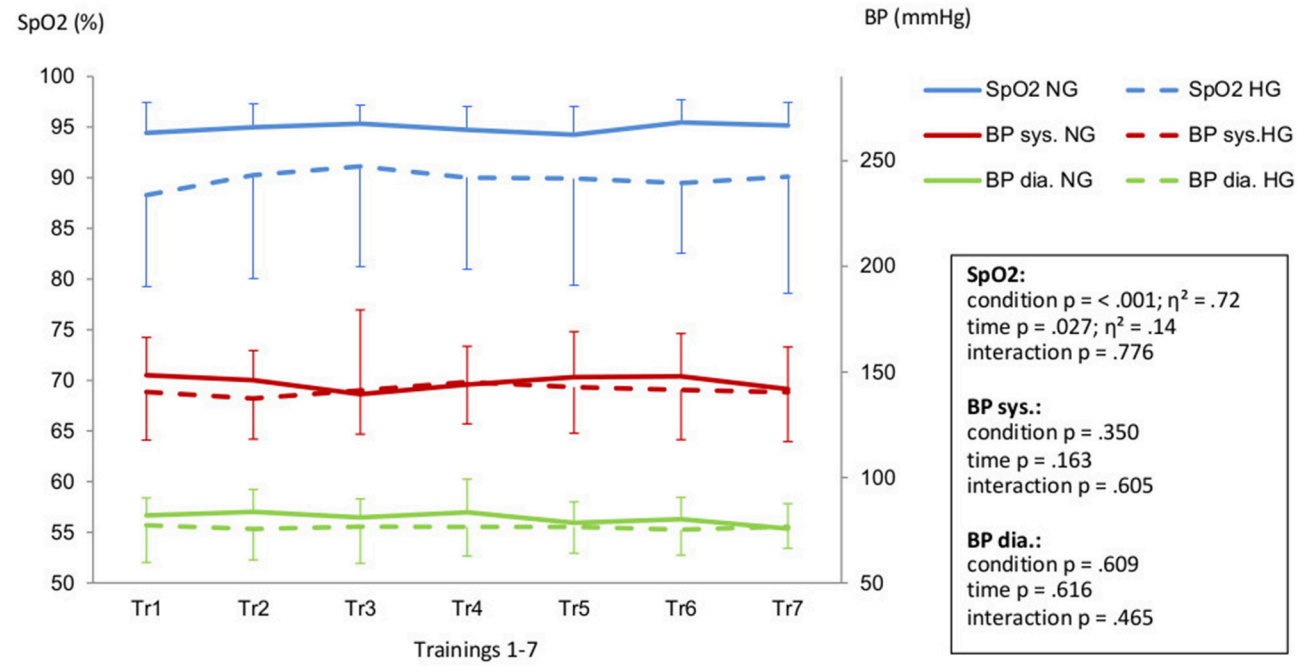

FIGURE 2 | Peripheral oxygen saturation (SpO_2) and systolic (BP sys.) aswell as diastolic blood pressure (BP dia.) in Hypoxic Group (HG) and Normoxic Group (NG) during the training intervention (Tr1-Tr7). Legend: Effect size is displayed as η^2.

for our population was not challenging enough to provoke measurable cardiac adaptations to the training. However, the endurance training reflected the routines commonly employed in geriatric rehabilitation programs. Therefore, it would not have been practical to prolong the training stimulus in our patients (Meusel, 2000). Rather than improving training stimulus (e.g.,

volume and/or intensity), we suggest that, depending of patients' health and fitness status, implementing longer "time in hypoxia" without training may be beneficial to provoke bigger changes in cardiac and pulmonary parameters. Alternatively, more frequent interventions, with sufficient regeneration time, during a single day may prove to be more effective (Bernardi et al., 2001; Vogt et al., 2002; Foster et al., 2005). More investigations are warranted using this specific population.

An increase in maximum power output was measured in both groups after the intervention. This finding is likely due in most part to the rehabilitation routine each subject underwent during their stay. The rehabilitation intervention included physical strength training, gait training, balance training and other common physical therapy methods. The relatively lower HR observed in HG during the post-test may be an early signal of an emerging higher stroke volume and improved O_2 utilization that has been demonstrated in other studies (Levine and Stray-Gundersen, 1997; Vissers et al., 2015; Lundby and Calbet, 2016; Moon et al., 2016). These positive outcomes may be attributed to the combined effects of the endurance training with an impactful target HR as well as the O_2 deprivation.

There are limitations to our study. The study was conducted in geriatric patients with a variety of primary diagnoses, multiple co-morbid conditions and complex medication regimens. These circumstances naturally influenced the outcomes of the study. Nevertheless, geriatric patients typically have complex medical conditions which cannot be standardized. The complex medical histories of our patient population is a strength of this study in that the results are more likely to pertain to usual clientele served by geriatric rehabilitation programs. As stated by the World Health Organization, several diseases that our health system aims to cure in young people can be seen as a disability when it comes to old age (WHO, 2001). In order to develop an applicable hypoxic endurance training program for geriatric patients, it is important not to exclude particular medical conditions or medications. Second, the rather short hypoxic protocol was not challenging enough to provoke significant cardiopulmonary adaptations in either randomized group. Third, a larger study population may have allowed for subset analyses to identify patients with particular characteristics that would be more responsive to this intervention. Fourth, the NG did not reach the estimated 80% VO_2 peak of training-intensity. The mean difference between NG and HG was 5.9 $\pm$ 4.2 bpm, which in our opinion does not influence the study outcome substantially. Nevertheless, the inability of the NG to reach the target training-HR validates the need for new methods to enhance training intensity.

In conclusion, the main outcome of this study demonstrated that endurance training in normobaric hypoxia demands less physical effort from the geriatric patient for the same degree of target HR response than similar training in normoxia. Our findings suggest that hypoxic endurance training is very likely to be more effective than normoxic training. Hypoxia has already been proposed to positively impact several common diseases in geriatric patients, such as hypertension, obesity, cognitive impairment and cardiopulmonary diseases (Burtscher, 2004; Millet et al., 2016; Schega et al., 2016). The great challenge for future investigations is to find a suitable and achievable training-protocol which provokes the expected beneficial adaptations for such population. Such a protocol should enhance geriatric rehabilitation while also taking into account the physical conditions that often limit the geriatric patients.

AUTHOR CONTRIBUTIONS

SP: designing research study, acquiring data, analyzing data, conducting experiment, writing manuscript. MB, MF and HG: writing manuscript, analyzing data. LR and AE: reviewing data, writing manuscript. NN: designing research study, conducting experiment, providing material, writing manuscript.

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